CANADIAN JOURNAL OF RESEARCH

VOLUME 27 APRIL, 1949 NUMBER 2

— SECTION D —

ZOOLOGICAL SCIENCES

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NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

CANADIAN JOURNAL OF RESEARCH

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For the present, Sections A, C, D, and E are to be issued six times annually, and Sections B and F, twelve times annually, each under separate cover, with separate pagination.

The *Canadian Journal of Research* is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. The *Canadian Journal of Research* is edited by a joint Editorial Board consisting of members of the National Research Council of Canada, the Royal Society of Canada, and the Chemical Institute of Canada.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 27, SEC. D. APRIL, 1949 NUMBER 2

A REDESCRIPTION OF *CEPHALOBELLUS BREVICAUDATUM* (LEIDY, 1851) CHRISTIE, 1933 (NEMATODA), WITH COMMENTS ON OTHER SPECIES OF THE GENUS *CEPHALOBELLUS*[1]

BY M. A. BASIR[2]

Abstract

A complete redescription of *Cephalobellus brevicaudatum* (Leidy, 1851) Christie, 1933, is given; its specific relationship is discussed, and a key to the species of the genus *Cephalobellus* is given. It is proposed that *Thelastoma indiana* Basir, 1940, and *Cephalobellus lloydi* Baylis, 1946, be regarded as synonymous with *C. brevicaudatum.*

In 1851 Leidy (8) described *Thelastoma brevicaudatum*, a new species of nematodes from the larvae of a "large lamellicorn insect." He gave no figures and his description was brief. He gave the following diagnosis:

> "Body white, cylindrical, anterior to commencement of ventriculus rapidly narrowed, conoidal; posteriorly abruptly rounded, with a very short spiculate tail. Generative aperture just posterior to middle. Anus just anterior to posterior rounded termination. Ventriculus broadly dilated at commencement, and moderately so at termination.
>
> "Length of body 1½ to 2 lines (=2.642-4.233 mm.); anterior to ventriculus 1/50 inch (=508μ); from anus to end of tail 1/200 inch (=127μ); at middle 1/80 inch (=317μ); at commencement of ventriculus 1/75 inch (=312μ); cylindrical portion of oesophagus 1/66 inch (=385μ) long by 1/666 inch (=51μ) broad; pyriform portion 1/250 inch (=101μ) long by 1/285 inch (=89μ) broad. Ovum semi-oval, 1/285 inch (=89μ) long by 1/1000 inch (=25μ) broad."

Later in the same year he (9) subdivided the genus *Aorurus* Leidy, 1849, into two subgenera, *Streptostoma* and *Thelastoma*, thus placing all the species previously described under the generic name *Thelastoma* into the genus *Aorurus*. *Thelastoma brevicaudatum* then became *Aorurus* (*Thelastoma*) *brevicaudatum.*

In 1853 the same author (10) raised the subgenera *Streptostoma* and *Thelastoma* to generic rank as *Streptostomum* and *Thelastomum*, and again gave a brief description of this species as *Thelastomum brevicaudatum.*

In the course of a synopsis of the parasites described by him up to that time, Leidy (11) placed this species as a queried synonym of *Thelastomum robustum*

[1] *Manuscript received November 26, 1948.*

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[The February issue of Section D (Can. J. Research, D, 27 : 1-29. 1949) was issued March 18, 1949.]

Leidy, 1850. Diesing (6) makes *Thelastoma* a subgenus of the genus *Anguillula* and calls *T. robustum*, *Anguillula* (*Thelastoma*) *robusta*, while retaining *T. brevicaudatum* as a synonym of *T. robustum*.

Travassos (12) suppressed the genus *Streptostomum* and revived the genus *Aorurus*, most probably on priority reasons. Walton (14) in his revision of the nematodes of the Leidy collections again revived the classification of Leidy (1851) and subdivided the genus *Aorurus* into two subgenera, *Streptostoma* and *Thelastoma*, as Leidy had done. Thus he again named this species *Aorurus* (*Thelastoma*) *brevicaudatus* (Leidy, 1851). Travassos (13) recognizes *Thelastoma* and *Aorurus* as distinct genera and calls this species by the name of *Thelastoma brevicaudatum* Leidy, 1851, as originally described by Leidy.

Christie (3) described *Scarabanema cylindricum* n.g., n.sp., from the larvae of several scarabaeid beetles. He removed *Thelastoma brevicaudatum* from the genus *Thelastoma* and placed it in his new genus as *S. brevicaudatum* n. comb. He also placed in his new genus, *Thelastoma leuckarti* (Hammerschmidt, 1838 (7)) Travassos, 1929, as *S. leuckarti* n. comb. Christie quotes the diagnosis of *S. brevicaudatum* from the original by Leidy and, in differentiating it from his species, remarks: "The above diagnosis taken from Leidy's original description indicates that this parasite belongs to the genus *Scarabanema*, but it is scant information upon which to identify the species." Later, in 1933, he (4) made his genus *Scarabanema* a synonym of the genus *Cephalobellus* Cobb, 1920, as he found that there was no difference between the male of *S. cylindricum* and *C. papilliger*. Cobb (5) has only given the description of the male worm unaccompanied by any figures. In 1946 Baylis added another species to this genus and named it *C. lloydi*.

The present writer recovered from a cockroach some worms, which appear to be identical with those described by Leidy as *C. brevicaudatum* (= *Thelastoma brevicaudatum*). As Leidy had given a very brief and incomplete description and had given no figures at all, it seems appropriate to take this opportunity to describe the species fully. No other worker, to the knowledge of the writer, has redescribed this species since Leidy, although Walton (14) reported that he had seen this form.

Cephalobellus brevicaudatum (Leidy, 1851) Christie, 1933

Synonyms:

Thelastoma brevicaudatum Leidy, 1851;
Aorurus (*Thelastoma*) *brevicaudatum* Leidy, 1853;
Thelastomum brevicaudatum Leidy, 1853;
Aorurus (*Thelastoma*) *brevicaudatus* (Leidy, 1851) Walton, 1927;
Scarabanema brevicaudatum (Leidy, 1851) Christie, 1933.
Thelastoma indiana Basir, 1940.
Cephalobellus lloydi Baylis, 1946.

Diagnosis: Cephalobellus

Male: Not found by the writer.

Female (Fig. 1): Body cylindrical, tapering more or less suddenly anterior to the base of the oesophagus, giving a conoid shape to the anterior end of the worm; posteriorly it tapers to form a short conoid spiculate tail. It is 2.2

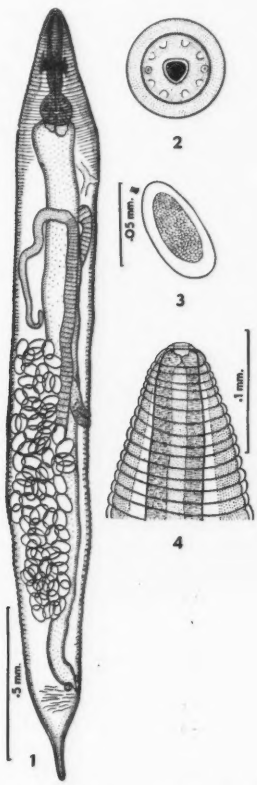

FIG. 1. *Female, entire lateral view.* FIG. 2. *Female, en face view.*
FIG. 3. *Egg.* FIG. 4. *Female, anterior region.*

to 2.6 mm. long by 250μ in maximum width. The cuticle is transversely striated throughout the whole length of the body except in the tail. The first annule behind the head is wider than the rest and is 13μ wide. The remaining annules, up to the middle of the body, are 8 to 12μ apart. In the posterior region of the body they are larger and reach a width of up to 15μ.

The oral opening is subtriangular and is surrounded by a circumoral elevation and eight papillae (Fig. 2). Amphids are present laterally as very small openings. The buccal cavity is short and is 8μ long by 8μ wide (Fig. 4).

The oesophagus nearly reaches the anterior extremity. It is 350 to 390μ long and consists of a more or less cylindrical corpus, 240 to 265μ long by 40 to 50μ wide, a short but distinct isthmus, 20μ long by 25 to 35μ wide, and a posterior valvular bulb 90 to 105μ long by 90 to 110μ wide. The oesophagus communicates with the intestine through a pair of well developed oesophagointestinal valves. The intestine is enlarged anteriorly to form a slight cardia. It also shows a posterior enlargement just before opening into the rectum. The rectum is short. Two rectal glands are present. The anus is situated 260 to 280μ from the posterior end of the body. The body suddenly tapers behind the anus and is drawn out into a short, blunt terminal spike.

The excretory pore lies posterior to the base of the oesophagus, about 600μ from the anterior end of the body. The nerve ring lies at about two-thirds of the length of the corpus from its anterior end, between 150 and 180μ from the anterior extremity.

The reproductive system is double. The vulva lies at a distance of 1.4 to 1.48 mm. from the anterior end of the body, 58 to 60% of the body length from the anterior end. The vagina is tubular and muscular and is directed anteriad. There are two ovaries. The anterior ovary is reflexed at a distance of about 280μ posterior to the base of the oesophagus and posteriorly the reproductive organs extend up to 250μ from the anus. The eggs are oval to elongated oval, 60 to 75μ long by 25 to 35μ wide, not segmented at time of deposition (Fig. 3).

Host: *Leucophoea* sp. (Family Blattidae).

Location: Intestine.

Distribution: North India; U.S.A. (near Philadelphia, Pa.).

Discussion

Those who have worked with the nematodes of the family Thelastomatidae know well that the characters of the male, which form the basic means for identifying genera and species of other groups, cannot be used for such a purpose in this group. Firstly, males are very rare and secondly, when they are found, there is often present a pronounced sexual dimorphism, so that in cases where two or more species inhabit the same host it is difficult to match the right males and females. Therefore, the basis of taxonomy of the Thelastomatidae rests mainly on the characters of the females.

Baylis (2) described *Cephalobellus lloydi* n. sp., from dipterous larvae (larvae of tipulids, crane-flies or "daddy longlegs") possibly belonging to *Tipula peliostigma* Schummel from Berkshire, and *Tipula oleracea* L. from Yorkshire (England). He compares his species with *C. papilliger*. The males he has described are almost identical with those described by Christie for the latter species, but his females are different from the females of that species in

that in *C. papilliger* the vulva lies at about 52% of the body length from the anterior end, and the eggs are subspherical to oval, 70μ long by 45μ wide; in *C. lloydi* the vulva is situated at about 61% of the body length from the anterior end, and the eggs are more than twice as long as broad, and are 82 to 96μ long by 32 to 38μ wide. In both these characters *C. lloydi* comes very close to *C. brevicaudatum*. The vulva in the latter lies between 58 and 60% of the body length from the anterior end and the eggs are 89μ long by 25μ wide. Baylis, in his discussion, writes: "The vulva, however, is stated by Leidy to be just posterior to the middle of the body, and this appears to differentiate the species from *C. lloydi*." As a matter of fact, Leidy did not give the exact position of the vulva. Now, in this present redescription of Leidy's species, the writer finds that the vulva lies at 58 to 60% of the body length from the anterior end. Thus this difference, on which Baylis mainly created his species, does not exist. The only difference now left is in the size of the eggs but if all the measurements, that is, those of Leidy, of Baylis, and of the writer are examined, it will be noticed that the sizes merge into each other.

Another difference, which Baylis points out, is in the respective hosts. Leidy found his worms in scarabaeid larvae. The worms described by Baylis were recovered from dipterous larvae while the writer obtained his from a cockroach. As the specificity of nematodes of arthropods has not yet been studied, it cannot be said that specific distinctions may be made on host relationship only, as has been done for forms like the different species of the genus *Strongyloides*.

As the morphology of both the worms is the same, the writer, after the present redescription, therefore, believes that *C. lloydi* Baylis, 1946, is synonymous with *C. brevicaudatum* (Leidy, 1851) Christie, 1933.

Thelastoma indiana Basir, 1940 (1), described from a cockroach, is also identical with *C. brevicaudatum* and, therefore, becomes a synonym to the latter species.

Thus the genus *Cephalobellus* Cobb, 1920, at present contains three species only, namely, *C. papilliger* Cobb, 1920; *C. leuckarti* (Hammerschmidt, 1838) Christie, 1933; and *C. brevicaudatum* (Leidy, 1851) Christie, 1933.

A revised key to these species is given below.

KEY TO THE SPECIES OF THE GENUS *Cephalobellus*

(1) Vulva almost in middle of body; eggs less than twice as long as broad..... 2

Vulva about 60% of the body length from the anterior end; eggs almost twice or more as long as broad..... *C. brevicaudatum*

(2) Eggs subspherical, 88–110μ long by 73μ wide; female with three conspicuous yellowish bodies near vagina..... *C. leuckarti*

Eggs smaller, oval, 70μ long by 45μ wide; females without any yellowish bodies near vagina..... *C. papilliger*

References

1. BASIR, M. A. Nematodes parasitic in Indian cockroaches. Proc. Ind. Acad. Sci., B, 12 : 8-16. 1940.
2. BAYLIS, H. A. A nematode parasite of tipulid larvae. Ann. Mag. Natural History, Ser. II, 13 : 53-59. 1946.
3. CHRISTIE, J. R. Some nemic parasites (Oxyuridae) of Coleopterous larvae. J. Agr. Research, 42 : 463-482. 1931.
4. CHRISTIE, J. R. The generic names *Cephalobellus* Cobb, 1920, and *Scarabanema* Christie, 1931 (Nematoda). J. Wash. Acad. Sci. 23 : 358. 1933.
5. COBB, N. A. One hundred new nemas. Contribs. Sci. Nematol. 9 : 217-343. 1920.
6. DIESING, K. M. Revision der Nematoden. Sitzungsber. K. Akad. Wiss. Wien, Math.-Naturw. Klasse, 42 (28) : 595-736. 1861.
7. HAMMERSCHMIDT, K. E. Beschreibung einiger Oxyuris-Arten. Naturw. Abhandl. 1 : 279-288. 1847.
8. LEIDY, J. Contribution to helminthology. Proc. Acad. Nat. Sci. Phila. 5 : 205-210. 1851.
9. LEIDY, J. Corrections and additions to former papers on helminthology. Proc. Acad. Nat. Sci. Phila. 5 : 284-290. 1851.
10. LEIDY, J. A flora and fauna within living animals. Smithsonian Inst. Pubs. Contribs. to Knowledge, 5 : 1-67. 1853.
11. LEIDY, J. A synopsis of Entozoa and some of their ecto-congeners observed by the author. Proc. Acad. Nat. Sci. Phila. 8 : 42-58. 1856.
12. TRAVASSOS, L. Esboco de uma chave geral dos Nematodes parasitos. Rev. Zootech. Vet. 59-71. 1919 (1920).
13. TRAVASSOS, L. Contribuicao preliminar a systematica dos nematodeos dos arthropodos. Mem. Inst. Oswaldo Cruz, 5 : 19-25. 1929.
14. WALTON, A. C. A revision of the nematodes of the Leidy collections. Proc. Acad. Nat. Sci. Phila. 79 : 49-163. 1927.

THE INFLUENCE OF GONADAL HORMONES ON THE SERUM VITAMIN A OF THE IMMATURE PULLET[1]

By D. G. Chapman,[2] M. Gluck,[3] R. H. Common,[4] and W. A. Maw[5]

Abstract

The serum vitamin A level of sexually immature pullets was increased threefold to fourfold by intramuscular injection of 24 mgm. estradiol dipropionate plus 4.5 mgm. testosterone propionate administered in six equal doses over a 12-day period as compared with the level in similar pullets receiving only testosterone propionate. It is suggested that gonadal hormone activity is one of the factors that regulate serum vitamin A levels in the domestic fowl.

Introduction

Administration of estrogens to sexually immature pullets or cockerels produces profound changes in blood composition. The resultant increases in serum calcium, serum inorganic phosphorus, serum phospholipid, and serum phosphoprotein have been extensively studied. The earlier literature has been reviewed by Riddle (9). Serum neutral fat and cholesterol are also increased (6), while there is some evidence that serum citric acid is decreased (3). Serum riboflavin is greatly increased (1), while the increase in blood volume appears to be due to an increase in plasma without a corresponding increase in volume of blood cells (2). There does not appear to be any extensive information as to the effects of estrogen on serum vitamin A in the fowl. Williamson (11) records that injections of estradiol benzoate decreased the serum vitamin A of rabbits. Gonadal hormone activity in the pullet as it comes into lay, however, almost certainly involves androgenic as well as estrogenic activity. The most obvious signs are the reddening and hypertrophy of comb and wattles that take place during the period of 10 to 14 days before laying. Treatments designed to simulate gonadal hormone activity in the pullet as it passes into the reproductive stage will therefore include an appropriate amount of androgen as well as estrogen.

So far as previous work goes it seems that estrogen alone will produce changes that simulate the relevant changes in serum calcium, serum phosphorus, and serum riboflavin. The magnitude of the changes evoked by estrogen in respect of these blood components is not affected by concurrent administration of androgen in amounts designed to cause changes simulating the relevant comb and wattle changes (1, 3, 4). Such concurrent administration of androgen does, however, enhance the effect of estrogen upon hypertrophy of the oviduct (1, 3, 4). Concurrent administration of androgen also enables estrogen to increase the rate of calcium retention so as to simulate

[1] *Manuscript received November 27, 1948.*

Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada. Macdonald College Journal Series No. 246.

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[5] *Professor of Poultry Husbandry.*

the increase in rate of calcium retention that precedes the normal beginning of laying (3, 4).

In the experiments now reported attention was restricted to the investigation of the effects on serum vitamin A of different levels of estrogen given concurrently with a single level of androgen. This inclusion of androgen was designed to secure a closer simulation of normal gonadal hormone activity than that obtained by estrogen treatment alone.

Experimental

Experiment 1

Eight Barred Plymouth Rock pullets of the same strain and hatching were placed in individual cages. They received a commercial starting mixture. Daily food consumption was kept equal as between the different birds. This point demands close attention in such experiments because of the stimulating effect of gonadal hormones on appetite. If fed *ad libitum*, then estrogenized birds consume more food and there is uncertainty as to the extent to which differential food consumption may be affecting the results.

The hormonal treatments were given in sesame oil solution by intramuscular injection into the breast muscles. Care was taken to ensure that the same total amount of sesame oil was injected into each bird.

Each treatment was divided into six equal doses administered on alternate days. All the pullets were treated with testosterone propionate (Schering) in amounts designed to secure a degree of comb hypertrophy comparable with that which takes place as the pullet comes into egg production. In addition graduated doses of estradiol dipropionate (Schering) were given as outlined in Table I.

TABLE I

EXPERIMENT 1. EFFECT OF GONADAL HORMONES ON SERUM VITAMIN A OF IMMATURE PULLETS

Pullet No.	1739	1743	1740	1744	1741	1745	1742	1746
Total dosage of estradiol dipropionate, mgm.	Nil	Nil	6	6	12	12	24	24
Total dosage of testosterone propionate, mgm.	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Live weight, initial, kgm.	1.12	0.99	1.05	1.00	1.06	1.15	1.24	1.10
Live weight, final, kgm.	1.27	1.09	1.23	1.16	1.18	1.23	1.34	1.21
Total food for experimental period, kgm.	0.83	0.83	0.84	0.85	0.84	0.84	0.84	0.84
Oviduct weight, gm.	0.24	0.30	13.9	16.2	17.0	20.9	22.4	17.2
Serum calcium, mgm./100 ml.	13.0	13.7	32.7	34.3	82.5	82.0	118.0	119.0
Crude blood volume, ml.	31.0	28.0	31.0	41.0	52.0	53.5	58.5	59.0
Crude blood volume, ml./kgm. live weight	24.4	25.7	25.2	35.4	44.1	43.5	43.6	48.8
Crude serum volume, ml./kgm. live weight	14.1	15.4	14.9	25.1	33.8	33.2	33.3	38.5
Serum vitamin A, I.U./100 ml.	153	119	170	282	320	499	528	602
Serum vitamin A, I.U./kgm. live weight	22	18	25	66	106	169	176	231

The birds were decapitated and bled on the second morning after the sixth injection. The blood was collected, allowed to clot, and the volume recorded as "crude blood volume". The "crude serum volume" per kgm. live weight

was calculated on the assumption that about 10.3 ml. cells per kgm. live weight will be obtained from such birds by free bleeding (2). The figures secured in this way are approximations, but they make it possible to arrive at some estimate of the total serum vitamin A per kgm. live weight.

Serum calcium was determined by Halverson's method (8). Serum vitamin A was estimated by the method of Dann and Evelyn (5), except that it was found necessary to use twice as much alkali in the initial saponification because of the higher protein content of the sera of estrogenized birds. The "L" values obtained with the Evelyn photoelectric colorimeter were converted to E values by using the factor 0.50 as determined by McFarlane and Sutherland (7). The E values were converted to I.U. vitamin A using the conversion factor 1894.

The blue values in the antimony trichloride reaction were corrected for carotene as described by Dann and Evelyn (5), but in all the experiments now described this correction was very small and could have been omitted without appreciably affecting the results.

The birds were 82 days old at the beginning of the injections and 94 days old when killed.

The data are summarized in Table I. The degree of hypertrophy of the oviduct and the increase in serum calcium and blood volume with increasing dosage of estrogen are similar to those recorded elsewhere for similar pullets (2, 4). There was not any evidence of ovarian activity.

The level of serum vitamin A was increased about fourfold by the heaviest estrogen treatment. The smaller treatments gave intermediate increases. The relative increase in serum vitamin A is considerably greater when considered on the basis of total serum vitamin A per kgm. live weight.

Experiment 2

This experiment was a repetition of Experiment 1, excepting that (*a*) three pullets were placed under each treatment and (*b*) the commercial starting mixture was replaced by the following mixture.

Ground wheat	25 lb.
Ground corn	25 lb.
Ground barley	25 lb.
Wheat middlings	5 lb.
Soybean oil meal	15 lb.
Fish meal	2.5 lb.
Salt	1.0 lb.
Calcium carbonate	2.0 lb.
Fortified fish oil (2200 I.U. vitamin A per gm.)	0.25 lb.
Manganese sulphate	6 gm. per 100 lb. mixture

The pullets were 84 days old at the beginning of the injections and 96 days old when killed.

The average results are given in Table II. It will be noted that they confirm the results secured in Experiment 1.

TABLE II

EXPERIMENT 2. EFFECT OF GONADAL HORMONES ON SERUM VITAMIN A; AVERAGE RESULTS FOR THREE PULLETS ON EACH TREATMENT

Total dosage estradiol dipropionate, mgm.	0	6	12	24
Total dosage testosterone propionate, mgm.	4.5	4.5	4.5	4.5
Live weight, initial, kgm.	0.98	0.92	1.00	1.04
Live weight, final, kgm.	1.17	1.11	1.19	1.15
Total food consumption, kgm.	0.72	0.76	0.74	0.72
Oviduct wt., gm.	0.26	17.8	18.8	22.6
Serum calcium, mgm./100 ml.	12.9	37.4	90.6	114.7
Crude blood volume, ml.	35	32	52	71
Crude blood volume, ml./kgm. live weight	30	29	47	59
Crude serum volume, mgm./kgm. live weight	29	25	37	49
Serum vitamin A, I.U./100 ml.	158	217	342	413*
Serum vitamin A, I.U./kgm. live weight	32	42	127	196*

**Omitting anomalous very low result for one serum.*

Experiment 3

A third experiment was carried out using 12 pullets, six of which received no estrogen and six of which received 18 mgm. estradiol benzoate (Schering). The birds received the same mixture as was fed in Experiment 2. The blood from one bird was accidentally lost. The average results for the other 11 pullets are set out in Table III.

TABLE III

EXPERIMENT 3. EFFECT OF GONADAL HORMONES ON SERUM VITAMIN A

	Group *A*	Group *B*
No. of pullets	5	6
Total dosage estradiol benzoate, mgm.	0	18
Total dosage testosterone propionate, mgm.	4.5	4.5
Live weight, initial, kgm.*	0.94	0.94
Live weight, final, kgm.*	1.11	1.09
Total food consumption, kgm.	0.78	0.79
Oviduct wt., gm.	0.22	14.8
Serum calcium, mgm./100 ml.	12.8	100.1
Crude blood vol., ml.	39.2	59.5
Crude blood vol., ml./kgm. live weight	35.8	44.6
Crude serum vol., ml./kgm. live weight	25.5	34.3
Serum vitamin A, I.U./100 ml.†	146	382
Serum vitamin A, I.U./kgm. live weight**	37	170

** Difference between groups not significant.*

*** Difference between groups highly significant at 1% level ("t" = 8.65).*

† Difference between groups highly significant at 1% level ("t" = 7.16).

The results leave no doubt that estrogen treatment superimposed on a small testosterone treatment increased the level of serum vitamin A.

Discussion and Summary

The levels of serum vitamin A observed in the unestrogenized pullets in these experiments fall well above the level of 50 to 75 I.U. per 100 ml. suggested by Taylor *et al.* (10) as representing marked depletion of body reserves. They are, in fact, of the same order of magnitude as the values found by Taylor *et al.* in the sera of hens receiving a diet judged adequate in provitamin A.

The increase in serum vitamin A that followed treatment with estrogen was not a consequence of increased food intake. The extra vitamin A must, therefore, have been obtained either (*a*) by increased retention from the food or (*b*) by depletion of body reserves or deflection of some absorbed vitamin A from storage. Taylor *et al.* (10) comment upon the variability of the serum vitamin A levels observed by them in laying hens receiving adequate provitamin A. It is possible that this variability is to some extent at least an expression of variability in endogenous estrogen production, which may control the distribution of vitamin A between liver reserves and the blood serum.

The present experiments leave little doubt that treatment with estrogen in conjunction with androgen increases serum vitamin A in the immature pullet. It seems reasonable to infer that the increase of endogenous estrogen and androgen production as the pullet comes naturally into reproduction will tend to affect serum vitamin A in the same sense.

Acknowledgments

The authors are indebted to Messrs. Schering & Company, Limited, Montreal, and to Messrs. Charles E. Frosst & Company, Montreal, for generous gifts of hormonal preparations. Part of the expenses was defrayed from a grant given by the House of Seagram, to whom the authors express their thanks. The financial assistance given by the National Research Council of Canada is also gratefully acknowledged.

References

1. COMMON, R. H. and BOLTON, W. Influence of gonadal hormones on the serum lipochrome and riboflavin of the domestic fowl. Nature, 158 : 95. 1946.
2. COMMON, R. H., BOLTON, W., and RUTLEDGE, W. A. The influence of gonadal hormones on the composition of blood and liver of the domestic fowl. J. Endocrinol. 5 : 263-273. 1948.
3. COMMON, R. H., RUTLEDGE, W. A., and BOLTON, W. Gonadal hormones and mineral balance in the domestic fowl. Nature, 160 : 541. 1947.
4. COMMON, R. H., RUTLEDGE, W. A., and HALE, R. W. Observations on the mineral metabolism of pullets. VIII. The influence of gonadal hormones on retention of calcium and phosphorus. J. Agr. Sci. 38 : 64-80. 1948.
5. DANN, W. J. and EVELYN, K. A. The determination of Vitamin A with the photoelectric colorimeter. Biochem. J. 32 : 1008-1017. 1938.
6. LORENZ, F. W., CHAIKOFF, I. L., and ENTENMAN, C. The endocrine control of lipid metabolism in the bird. II. The effects of estrin on the blood lipids of the immature domestic fowl. J. Biol. Chem. 126 : 763-769. 1938.
7. MCFARLANE, W. D. and SUTHERLAND, A. J. Study of the determination of vitamin A by spectrophotometry and by the photoelectric colorimeter. Can. J. Research, B, 16 : 421-431. 1938.

8. PETERS, J. P. and VAN SLYKE, D. D. Quantitative clinical chemistry. Vol. 2. Baillière, Tindall & Cox, London. 1932.
9. RIDDLE, O. Cyclic changes in blood calcium, phosphorus and fat in relation to egg laying and estrogen production. Endocrinology, 31 : 498-506. 1942.
10. TAYLOR, M. W., STERN, J. R., RUSSELL, W. C., and JUNGHERR, E. The provitamin A requirement of hens. Poultry Sci. 26 : 243-254. 1947.
11. WILLIAMSON, M. B. Effect of estrogen on plasma vitamin A of normal and thyroidectomized rabbits. Proc. Soc. Exptl. Biol. Med. 66 : 621-623. 1947.

THE STRUCTURE OF THE ASCIDIAN, *PYCNOCLAVELLA STANLEYI* N. SP., AND THE NATURE OF ITS TADPOLE LARVA[1]

By N. J. Berrill[2] and D. P. Abbott[3]

Abstract

A new species of an ascidian, *Pycnoclavella stanleyi*, is described. It occurs in some abundance near low tide level at Pacific Grove, Calif. It differs from *P. aurilucens* Garstang, known only from the western end of the English Channel, mainly in the character of the colony rather than that of the individual zooid or tadpole larva. Colonies are flat, incrusting, heavily impregnated with sand, have a gelatinous matrix, and exhibit some orange pigmentation. Colonies of *P. aurilucens* are without sand, have a leathery texture, are green, and are not intertidal. Both species have zooids with seven rows of gill slits on each side, a posterior vascular budding stolon as in *Clavelina*, and a tadpole larva with an ocellus but not an otolith.

In his account of the ascidian *Euherdmania claviformis*, Van Name (9) states that "at various points on the California coast (at Pacific Grove and Bodega Bay) there may be found among the roots of seaweeds near low-water mark, where sand as well as rocks are present, dense sandy masses of small tubular zooids closely packed, the masses often 6 to 10 cm. across, which appear to be colonies of young zooids of this species." He found the colonies to be immature and doubted that they could be a form allied to *E. claviformis* but with smaller zooids, yet felt that the matter deserved further study.

Collections of ascidians have recently been made by one of us (D.P.A.) in the region of Pacific Grove. Ascidian colonies with the general appearance of those described by Van Name were found at Aselomar Rocks and at other points. Some of these contained zooids with but three rows of gill slits and appear to be colonies of *Eudistoma ritteri* Van Name. Other colonies had zooids with seven rows of gill slits, a number that excludes them from the genus *Eudistoma* and almost as certainly from *Euherdmania* as well. When the character of the gills is taken together with other characters, no doubt is left that the colonies are a species of the genus *Pycnoclavella*, established by Garstang in 1891, and that the species is new. It is a pleasure, therefore, of the senior author to name it *Pycnoclavella stanleyi* after his colleague, John Stanley. This accordingly becomes the second species of the genus, the other and type species being *Pycnoclavella aurilucens* Garstang (8), recorded with certainty only near Plymouth, from the Mewstone ledge in the English Channel; the structure and life cycle of *P. aurilucens* have been recently described elsewhere (1).

[1] *Manuscript received November 13, 1948.*

Contribution from the Department of Zoology, McGill University, Montreal, Que., and the Department of Zoology, University of California, Berkeley, Calif.

[2] *Professor of Zoology, McGill University.*

[3] *Instructor in Zoology, University of California.*

Pycnoclavella stanleyi n. sp.

The colonies grow on rocks or at the base of seaweeds near the low-water mark, forming flat masses several centimeters across and about 1 cm. thick. Except for the thoracic region, individual zooids are usually embedded in the common test, which is comparatively soft, and the thoracic part of each zooid extends freely from the exposed surface. Each colony examined showed some evidence of being superimposed upon hydroid creeping stolons, and it is possible that these are a factor in its initial establishment. Two superficial characters are distinctive. The matrix of the colony is impregnated and covered with sand grains of all kinds, together with small worm tubes and calcareous algae, making observations of the basal regions most difficult, while the emergent thoracic regions are typically a bright orange, a color that fades upon preservation to a dull brown. The orange color is due to special cells scattered through the test surrounding the free part of each zooid outside the epidermis. The function of these cells is obscure.

Structure of zooid

The general morphology of the zooid is shown in Fig. 1. The zooid is divisible into a thorax and abdomen, with an attenuated oesophageal region between. There is no true postabdomen as in *Euherdmania*. Each zooid, being an independent unit, has separate branchial and atrial siphons. There is no arrangement of zooids into systems and consequently no corresponding modification of the atrial siphon. Both siphons are smooth-edged, as in *Clavelina* and *Euherdmania*, and not divided into six lobes as in *Eudistoma*.

The branchial sac has seven rows of stigmata on each side, in contrast to the three rows of *Eudistoma* and 10 rows of *Euherdmania*. The abdomen is long and slender, with the digestive tube descending to its posterior limit. The digestive tube itself consists of a long narrow oesophagus opening posteriorly into a more or less globular stomach in the lower part of the abdomen, a mid-intestine forming the posterior loop proper, connecting the stomach with the long ascending rectum, which opens at the base of the atrial cavity (Fig. 1A). The rectum is commonly distended like a string of beads by the large round faecal pellets formed in passage through the stomach.

The heart and pericardium lie alongside the posterior loop of the intestine, and has one end opening into the ventral vessel that extends forwards eventually to pass along the ventral side of the endostyle, and posteriorly to open towards the posterior vascular extension (Fig. 1A, *pv*). This last structure is usually short and narrow and contains an internal vascular septum separating the afferent and efferent flow of the blood. At the junction of this extension with the abdomen are three corrugated protrusions representing the posterior insertions of the longitudinal muscles of the body.

The epicardium, i.e. the closed membranous sac adjoining the digestive tube, of possible excretory function, extends as a simple single sac flattened dorso-ventrally, from just below the thorax to the lower part of the heart, at the ventral side of the digestive tract (*cp*, cross sections shown in Fig. 1, B, C, and D).

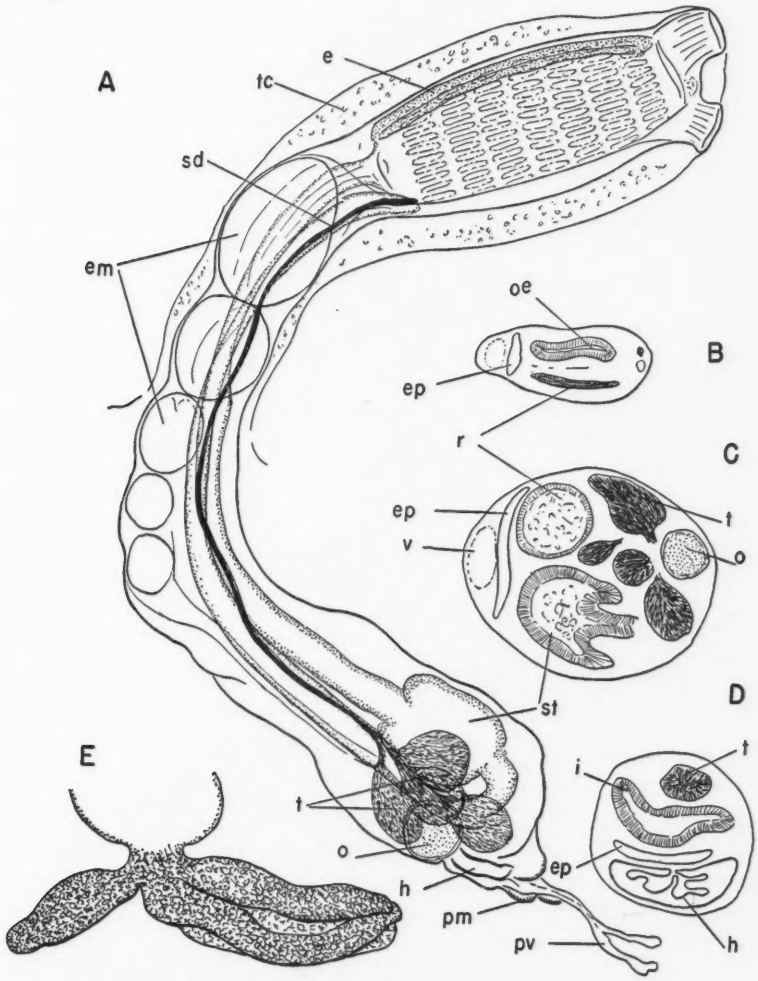


FIG. 1. *Structure of zooid of Pycnoclavella stanleyi.*

A, complete zooid showing thorax with independent siphons and seven rows of gill slits, oesophageal region with developing embryos, and abdomen with stomach, gonads, and heart. B, C, D, cross sections of zooid at level of oesophagus, stomach, and heart, respectively. E, posterior end of zooid in accumulation phase, with posterior vessels distended with reserve cells.

e, endostyle; em, embryos; ep, epicardium; h, heart;, i, loop of intestine; o, ovum; oe, oesophagus; pm, posterior muscle insertions; pv, posterior vessel; r, rectum; sd, sperm duct; st, stomach; t, lobe of testis; tc, test cell; v, ventral vessel.

The gonads lie in the abdomen at approximately the same level as the stomach and consist of an ovary and several testicular lobes, the oviduct and common sperm duct following the course of the rectum to open at the base of the atrial cavity.

Eggs are passed from the ovary one at a time into the oviduct as they ripen and are there fertilized, mature zooids like the one illustrated usually possessing a series of embryos developing within the oviduct, which thus serves as a brood chamber.

Budding

The only colonies available for examination were collected in July during the sexual breeding period, and ascidian colonies in general rarely exhibit budding processes and sexual reproduction simultaneously. At the same time two colonies showed an unusual condition at their base. The posterior region of the zooids of these colonies was extended and distended, in the majority involving the posterior vascular extension only, in some the posterior end of the abdomen including the longitudinal muscles. A typically inflated region is shown in Fig. 1E. The vascular processes especially are distended with reserve cells. These are large cells heavily laden with several large pseudo-yolk granules and corresponding to the trophocytes of *Clavelina* (7) and *Diazona* (4). These cells are to be found scattered through the body of the zooid and they migrate posteriorly and progressively accumulate in the lower part of the abdomen. With dilatation of the posterior extremity and vascular processes, they pass into the processes to form massive accumulations of reserve material. They appear to be similar to, and possibly may be identical with, the orange cells of the thoracic test.

The structure of the zooid is such that only two methods of budding appear to be possible. The abdomen, including the oesophageal region, may undergo transverse epidermal constrictions to form several buds, as in *Eudistoma* and *Archidistoma* (2, 3), or the zooid as a whole may regress, leaving the congested posterior vascular processes to give rise to new zooids as in species of *Clavelina* (6) and as is probable in *Pycnoclavella aurilucens* (1). The latter is much the more likely, for it is only in those forms in which the reserve cells congest the abdomen that abdominal buds are formed. When the reserve cells can pass posterior to the abdomen into either distended vascular processes or into a true postabdomen, both abdomen and thorax undergo regression, and since this species allows this passage to take place, this probably occurs.

Nature of Tadpole Larva

The structure of the tadpole is shown in Fig. 2. The fully formed tadpole (Fig. 2A) is relatively large, with an ovoid trunk and comparatively long tail. The tail is twisted at its base so that its axis is at right angles to the axis of the trunk. There are the usual three anterior adhesive organs (Fig. 2A, *ad*), while the tail structure is primitive, with three rows of muscle cells along each

side of the central notochord (Fig. 2E). The primary ascidiozooid is well formed within the trunk and has its main anteroposterior axis already at right angles to that of the tadpole itself.

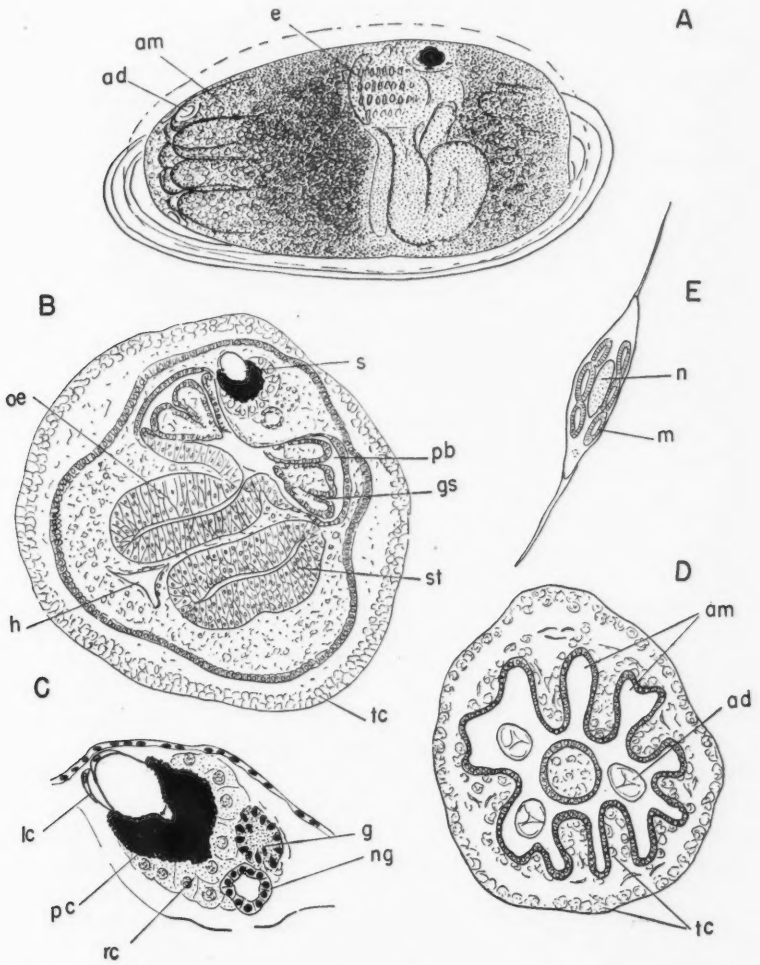


FIG. 2. *Tadpole larva of Pycnoclavella stanleyi.*

A, whole tadpole with tail coiled, showing single sense organ, anterior ampullary structure, and central ascidiozooid with four rows of gill slits. B, transverse section through level of sense organ. C, sense organ enlarged to show optic cup and lens cells. D, transverse section through anterior ampullae. E, transverse section of tail.

ad, adhesive organ; am, ampulla; e, endostyle; g, neural ganglion; gs, gill slit; h, heart; lc, lens cell; m, tail muscle cell; n, notochord; oe, oesophagus; pb, peribranchial cavity; pc, pigment cup; rc, retinal cell; s, sense organ; st, stomach; tc, test cells.

There are, however, three distinctive features. The test substance surrounding the trunk is heavily impregnated with large pigmented test cells derived from the tadpole and not from the parent zooid (Fig. 2B, D, *tc*). The anterior epidermis of the tadpole is developed to form about eight ampullary divisions (Fig. 2A, D, *am*), while the sensory vesicle contains an ocellus only, there being no trace of an otolith.

There is virtually no free space within the sensory vesicle. The neural ganglion and gland of the future zooid are already well developed (Fig. 2C, *g*, *ng*). The ocellus itself consists of a typical pigment cup partly surrounded by a single layer of large retinal cells, and two large lens cells (Fig. 2C, *lc*).

Discussion

This species shares with *Pycnoclavella aurilucens* the following features,—thorax of zooids extending freely from a common matrix, siphons smooth-edged, branchial sac with seven rows of gill slits on each side, no postabdomen, posterior vascular processes becoming distended with reserve cells, embryos developing in series within oviduct, and tadpole larva with ocellus but no otolith. These features in common are more than sufficient to unite the two forms as species of the same genus, and the absence of an otolith in the tadpole becomes here a generic and not a specific character.

Euherdmania zooids have 10 or more rows of gill slits, a true postabdomen containing heart and gonads, a uniquely double epicardium, and a tadpole with both ocellus and otolith (5). There is consequently no possibility that we are dealing with a species of this genus.

The differences in the two colonies refer mainly to details of the common test or matrix in which the zooids are embedded. In *P. aurilucens* the test cells are green instead of orange and there is no tendency to incorporate sand grains in its comparatively leathery test. The tadpoles differ in one feature only, namely, the presence of the ampullary organization of the anterior trunk epidermis of *P. stanleyi* and the absence of this in *P. aurilucens*. Anterior ampullae are so frequent among the various ascidian tadpoles that it is hardly distinctive, though in this case it does suggest some affinity with the *Archidistoma–Eudistoma* forms rather than with *Clavelina* itself.

The distribution of these two species is of interest. Each is known only from a very limited locality, for there is no record of *P. aurilucens* other than from one or two localities in the western end of the English Channel, while *P. stanleyi* is known from an equally limited region of the North American Pacific coast.

References

1. BERRILL, N. J. The structure, tadpole and budding of the ascidian *Pycnoclavella aurilucens* Garstang. J. Marine Biol. Assoc. United Kingdom, 27 : 245–251. 1947.
2. BERRILL, N. J. The structure, development and budding of the ascidian, *Eudistoma*. J. Morphol. 81 : 269-281. 1947.
3. BERRILL, N. J. Structure, tadpole and bud formation in the ascidian *Archidistoma*. J. Marine Biol. Assoc. United Kingdom, 27 : 380-388. 1948.

4. BERRILL, N. J. The development, morphology and budding of the ascidian *Diazona*. J. Marine Biol. Assoc. United Kingdom, 27 : 389-399. 1948.
5. BERRILL, N. J. Tadpole larvae of the ascidians *Polycitor*, *Euherdmania* and *Polysyncraton*. J. Morphol. 82 : 355-364. 1948.
6. BERRILL, N. J. and COHEN, A. Regeneration in *Clavelina lepadiformis*. J. Exptl. Biol. 13 : 352-356. 1937.
7. BRIEN, P. and BRIEN, E. Contributions à l'étude de la blastogénèse des Tuniciers bourgeonnement de *Clavelina lepadiformis* Muller. Rec. Inst. zool. Torley-Rousseau, 1 : 31-81. 1927.
8. GARSTANG, W. Report on the Tunicata of Plymouth. J. Marine Biol. Assoc. United Kingdom, 2 : 47-67. 1891.
9. VAN NAME, W. G. North and South American Ascidians. Bull. Am. Museum Nat. Hist. 84. 1945.

THE TWO TYPES OF EPITHELIUM OF THE FINEST BRONCHIOLES OF THE ALBINO MOUSE AS REVEALED BY SUPRAVITAL SILVERIZATION[1]

By Charles C. Macklin[2]

Abstract

Two definite types of cells, provisionally designated dark and light, in accordance with the degree of their superficial argyrophilism, are revealed in the terminal bronchiolar epithelium of the albino mouse by supravital silverization. The dark cells are ciliated, and scattered among the light unciliated cells as singles, pairs, triads, or larger groups to form a striking and characteristic surface pattern. The cuticles of the dark cells, far outreached by the neighboring light cells, appear *en face* as dense crowds of small uniform golden brown granules. Viewed laterally these show as pairs in vertical alignment, and make two layers with a thin lighter band between. The sides and bases are indicated by deposits of black grains. Near the sharply marked margin of the definitive epithelium the dark cells are shorter, more dispersed, and formed like truncated pyramids with narrow densely impregnated apices on some of which are single delicate points instead of discrete cilia. The light cells have bulging villuslike free ends and make flutings on the contiguous dark cells. Small silverized particles sparsely stipple their air surfaces and rounded plaques crown their summits. A similar dicellular picture is found in the terminal bronchiolar epithelium of the golden hamster.

Introduction

The epithelium of the last quarter millimeter of the purely conducting bronchioles (Macklin, 1929 (4)) is largely unexplored. In an attempt to learn more about it the following experiments, mainly with healthy adult albino mice, were carried out. Two distinct and highly differentiated types of epithelial cells were made to stand out from one another by washing them while still alive with an ammoniacal silver solution. Only one of these is ciliated. The morphology and function of the other cell type—unciliated and much more conspicuous—are not well understood.

Material and Method

Each animal was anesthetized lightly with ether, the abdominal cavity opened, and the great vessels cut to exsanguinate the lungs. The diaphragm was carefully pinched up with fine forceps and nicked. With a hypodermic syringe inserted into the exposed trachea the now collapsed lungs were filled with silver solution to about the stage of full inspiration. After two minutes it was drained away, and the lungs were at once refilled with 10% formalin. The trachea was tied off, and the lungs and heart promptly removed and

[1] *Manuscript received in original form November 23, 1948, and, as revised, January 20, 1949.*

Contribution from the Department of Histological Research, Faculty of Medicine, the University of Western Ontario, London, Ont. A précis of this paper was kindly read by Prof. W. J. Hamilton of Charing Cross Hospital Medical School, the University of London, at the Summer Meeting of the Anatomical Society of Great Britain and Ireland on June 25th, 1948, in the University of Bristol.

[2] *Research Professor of Histology.*

placed in a bath of 10% formalin in which they remained for a day or more, standing exposed to daylight and at times direct sunlight. Frozen and paraffin sections were made, some of which were treated with direct sunlight and others with weak photographic developer. Many of the frozen sections were dried and flattened on the slide, and these were useful in looking over reaches of terminal bronchiolar epithelium. The paraffin sections were cut both thin and thick. All sections were examined clear, or after staining in different ways. The ammoniacal silver solution (Hodgman and Lange, 1930 (2)) was made up as follows:

Solution 1

Five grams of silver nitrate was dissolved in 300 cm.^{3} of distilled water and dilute *aqua ammoniae* was added until the precipitate formed was nearly but not entirely redissolved. It was then filtered, and water was added to make 500 cm.^{3}

Solution 2

One gram of silver nitrate was dissolved in a small quantity of water and poured into half a liter of boiling water. Rochelle salt (0.83 gm.) was dissolved in a small quantity of water and added to the boiling solution, which was then boiled for half an hour till a gray precipitate collected as a powder in the bottom of the flask. It was then filtered hot, and water was added to make 500 cm.^{3}

In using, equal parts of the two solutions were mixed. The separate solutions, kept in the dark, remain good for a month or two. Minor variations of composition were tried out, with approximately similar results.

Histological Observations

The low-power bird's-eye view (Fig. 1) of a typical cleared strip of silverized terminal bronchiole *t b* from a flattened frozen section is striking, being a mixture of sharply contrasting dark and light areas. Traced to the right the dark spots become less and less conspicuous, more scattered, and smaller, as we pass into the region of the alveolated duct at *a d*. A respiratory bronchiole in the usual sense is often difficult to discern in the mouse. As the intervening light areas are in a similar way traced to the right they come to occupy more and more of the presenting surface. At the left of the figure is part of the contour of a larger bronchiole *b*, and its surface also has a mottled appearance. The dark islands and tracts are the air surfaces of the silverized cells and the light ones are those of the relatively unsilverized cells. These are hereinafter called the dark and light cells, respectively. The dark cells are somewhat smaller in surface area than the others and are ciliated. The curious light or clear cells have protruding egg-shaped ends. They may be classified generally as columnar, though differing greatly from ordinary columnar epithelial cells.

They possess an individuality in high degree. The delicate cross striations in the middle of Fig. 1 are due to silver impregnation of the endothelium of the transverse bronchiolar pulmonary arteriole, *a*.

Fig. 2 is an oil immersion photomicrograph from the middle of a similar strip. The dark and light cells contrast strongly. The dark cells are more heavily outlined by silver deposits, and their surfaces are densely and fairly evenly covered with fine brown silverized granules of uniform size. The clear-cut cell contours are angulated, and the dark silver lines joining the projections are often incurved. The dark cells here occur singly or in groups of two, three, or more. The focal plane corresponds with the general level of the surfaces of the dark cells, which lie below the level of the apices of the domes of the light cells. No afterstaining was done, so that the nuclei and other details are not seen. The borders of the light cells are very imperfectly indicated by silverized granules. The contours are not acutely angulated, but rather tend to approach the circular form. The fact that they bulge laterally into the dark cells suggests that their protoplasm is of a firmer nature. Since their

Explanation of Figures

All figures are photomicrographs from the mouse lung except No. 14, which is from the lung of the golden hamster. All figures except two show only the effects of the supravital silverization, there having been no afterstaining. The exceptions are Nos. 12 and 13, which had no silver treatment, and were stained with iron haematoxylin. The technical details are given in the text.

Abbreviations:

a – *transverse bronchiolar pulmonary arteriole*
a d – *alveolated duct*
b – *bronchiole*
d c – *dark cell*
du c – *dust cell*
e b – *epithelial borderland*
j – *approximate point of junction of the paired terminal bronchioles shown*
t b – *terminal bronchiole*

Magnifications:

×180 – *Figs. 1, 5, and 6.*
×790 – *Figs. 7, 8, and 12.*
×1800 – *Figs. 2, 3, 4, 9, 10, 11, 13, and 14.*

Plate I

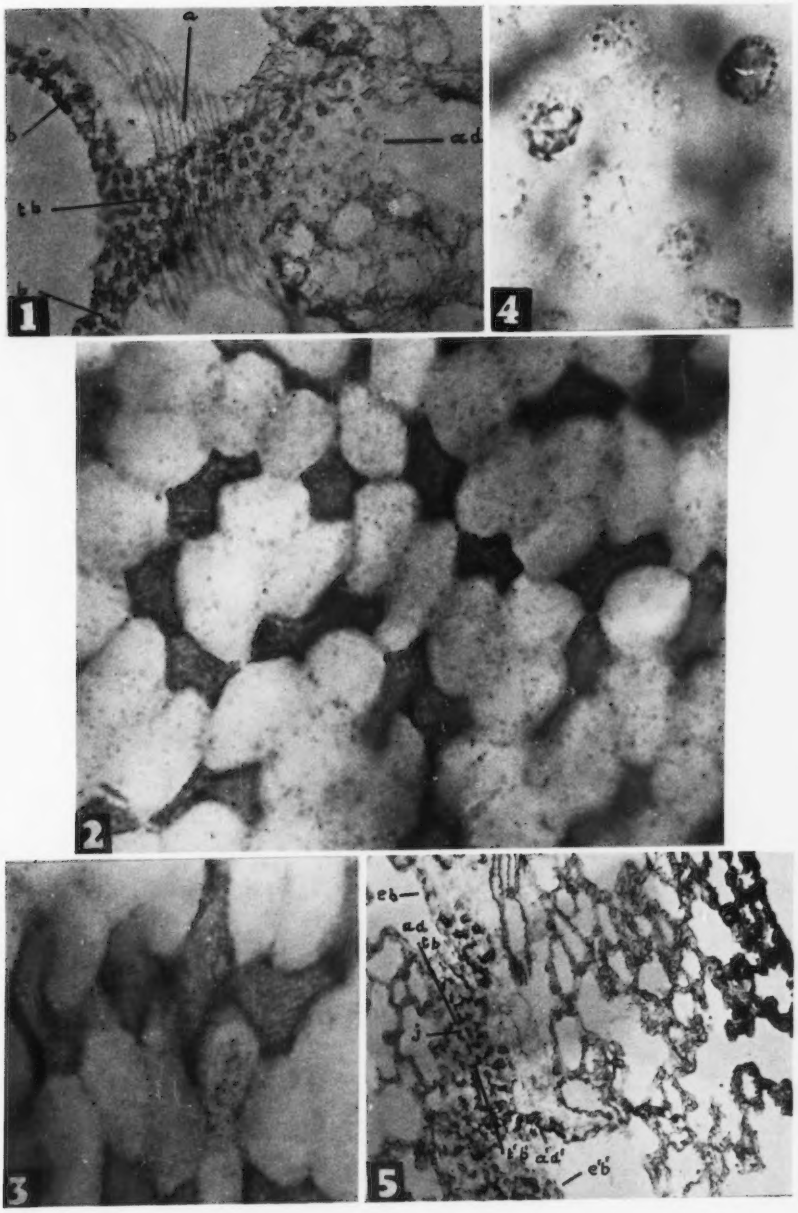
Fig. 1. *Flattened frozen section at 30μ of a supravitally silverized lung, without afterstaining. It shows the surface pattern of dark and light cells in the epithelium of a terminal bronchiole. See text for description. S92-18Ms-3.*

Fig. 2. *A small area from a field like that of Fig. 1, highly magnified. The finely granular faces of the dark cells and their sharply marked curved contours are outstanding features. S92-17Ms-1.*

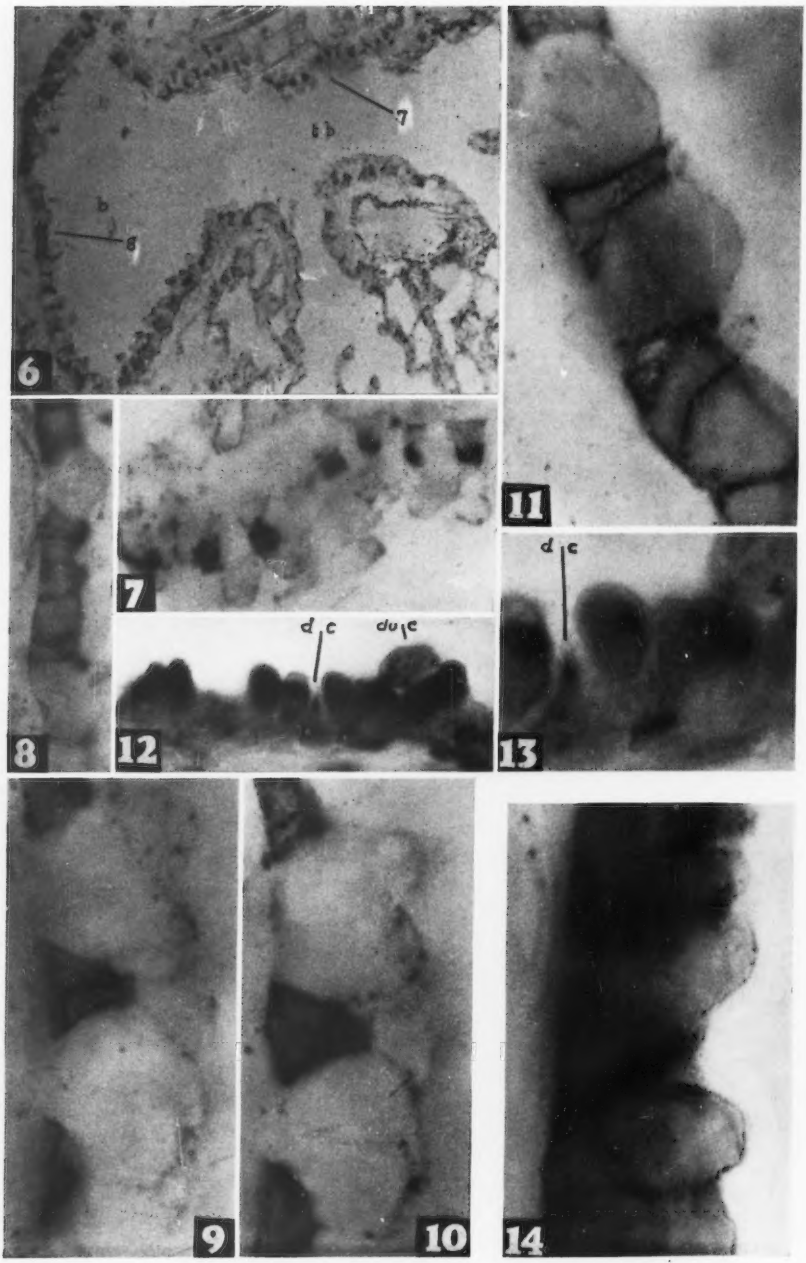
Fig. 3. *The design and surface details of the dark and light cells of a terminal bronchiole in a flattened frozen section of 30μ, unstained, at high magnification, are seen. S92-17Ms-1.*

Fig. 4. *A high-level view of part of the field of Fig. 2 to give en face views of the granular plaques surmounting the domes of the light cells.*

Fig. 5. *The divaricated terminal bronchioles t b, t′ b′, are merged at j and present, in a 10μ paraffin section, a strip of their epithelium in which the contrasting dark and light cell formations can be traced opposite to the regions of their respective alveolated ducts, a d and a′ d′. Attenuation of the dark cell pattern toward the epithelial borderland e b, e′ b′ is seen. S92-14Ms-8.*



FIGS. 1 TO 5.



FIGS. 6 TO 14.

tips, rising toward the eye of the observer, lie at a higher level than the focal plane of the photograph, they are not visible. By raising the objective, scattered fine dark granules are seen upon their egg-shaped domes; and surmounting the majority of these are discoid areas that contain more numerous granules. Fig. 3 is an oil immersion view of another surface of terminal bronchiolar epithelium. The boundaries between the light cells are marked by rows of black dots, and the surface stippling of the dark cells may be seen. The rounded plaques surmounting the domes of the light cells shown in Fig. 2, appear in an oil immersion surface view in Fig. 4. In some of these a fairly complete ring of small and intensely dark granules is on or just within the periphery. It is often difficult to distinguish the boundary of the disk from the contour of the underlying dome. The significance of these plaques is unknown.

In Fig. 5 we have a view at low power of part of the confluential walls of two terminal bronchioles, *t b*, *t' b'*, pointing in opposite directions to their respective alveolated duct systems, *a d* and *a' d'*. The approximate junctional point *j* is presumably opposite the supply bronchiole, which is not included in

PLATE II

(For general explanation of figures see p. 52)

FIG. 6. *Profile views of dark and light cells in a bronchiole b and a terminal bronchiole t b are seen in a 10μ cleared paraffin section. At 7 and 8 are indicated small lengths of silverized epithelium, which are more highly magnified in Figs. 7 and 8, respectively. S92-14Ms-8.*

FIG. 7. *The dark cells in this terminal bronchiole are short and often pyramidal in shape. Their dense cuticular parts are lowermost. Between them are the light cells, whose protruding ends are imperfectly outlined in fine silvered granules.*

FIG. 8. *Dark and light cells from the wall of bronchiole b, Fig. 6, are seen. The dark cells tend to be in larger groups, and the light cells lower, than in the terminal bronchiole, Fig. 7.*

FIG. 9. *A highly magnified view of silverized terminal bronchiole epithelium is seen in this 10μ paraffin section. The middle cell, a truncated pyramid, has densely silvered edges. Its contracted apex is dark golden brown in color. S92-14Ms-8.*

FIG. 10. *The dark cells of Fig. 9 are seen at a lower focal level. The narrow cuticular air face of the middle cell is distinct at the bottom of a funnel-shaped depression, hedged in by the projecting ends of the light cells.*

FIG. 11. *A short length of epithelium from a bronchiole larger than that of Fig. 6, b is seen in this 10μ paraffin section. The sharply outlined, relatively high dark cells have flaring bases and bear tufts of cilia on their narrow cuticular surfaces. The spheroidal light cells press laterally into the dark cells, and have low domes projecting into the lumen. S92-14Ms-8.*

FIG. 12. *In this unsilverized material, fixed in Bouin's fluid, cut at 10μ from paraffin and stained with iron haematoxylin and eosin, the dark cells, low and inconspicuous, might easily be overlooked. One of them , d c, shows a sharp apex. The rounded projections of the light cells here stain densely. S92-23Ms-2P-2.*

FIG. 13. *This is a high magnification of a part of Fig. 12.*

FIG. 14. *In this section of silverized lung from the golden hamster the fundamental characteristics of silver impregnation, and of the morphology of the dark and light cells, are the same as those of the mouse. In this terminal bronchiolar epithelium the dark cells are lower than are the light cells, and their narrow cuticular areas, bearing cilia, are flanked by the bulging ends of the light cells. S92-15H-1, paraffin section, 25μ.*

this section. The pattern of dark and light areas is typical. As in Fig. 1, the dark cell faces become less conspicuous toward the alveolated ducts. Some side views of dark cells are imperfectly visible in the epithelial borderland *e b*, *e' b'*. Low-power profile views of bronchiolar and terminal bronchiolar epithelium after supravital silverization are given in Fig. 6. To the left is a bronchiole *b* leading into a terminal bronchiole *t b* cut lengthwise. The dark and light areas representing the two types of cell of the epithelial coat are plainly seen. Owing to unevennesses in the epithelial layer, the cells are not always cut so as to present their long axes exactly in the plane of the section. In the wall of the bronchiole *b* the dark cells are larger and more regular than they are in the terminal bronchiole. The numerals 7 and 8 indicate the areas of epithelium shown in Figs. 7 and 8, respectively, at higher magnification. The dark cells seen in Fig. 7 appear short and tend to be triangular in outline. The air surfaces face downward. The air face of each dark cell is formed by a mass of somber brown silverized material. The cell outlines are indicated by layers of fine black granules. The protuberances of the light cells, extending downward below the levels of the apices of the contiguous dark cells, are faintly outlined in sparse fine dark granules. The air surface in Fig. 8 faces the right: here the dark cells have a more regular outline and occur in larger groups. Their exposed faces, strongly marked in dark brown silver deposit, are relatively broader and bear granules whose linear arrangement suggests that it has been determined by the presence of cilia. The boundaries of these faces are, as before, outlined by numerous small granules. The light cells, unciliated, do not project quite as prominently as in the terminal bronchiolar epithelium of Fig. 7.

The typical pyramidal shape of a dark cell situated near the margin of a terminal bronchiole is seen in the oil immersion view from another region of this section (Fig. 9). The other dark cells above and below in this field are not axially in focus. The very narrow apex appears dark brown and is almost buried between the towering ends of the contiguous clear cells. The granules marking the sides and broad base are probably a precipitate of silver, and are black, rather than brown. The angle lines of the sides and base are very strongly pencilled. These cells appear in Fig. 10 at a deeper focal level. For comparison with the foregoing we have in the oil immersion view of Fig. 11 the dark and light cells from a larger bronchiole of the same section. The dark cells are here somewhat higher and bear granular formations suggesting definite tufts of cilia. The walls of the dark cells are typically incurved, presumably from contact with the light cells, and their bases are often flaring. As before, their outlines are marked by fine black granules of silver. In the conspicuous golden brown cuticle an inner and an outer thin layer of darker material, separated by a thin layer of lighter substance, can be made out in the section, but not clearly in the figure. These darker layers can be resolved into brown granules in vertical pairs, apparently basally related to the cilia.

The light cells are here more nearly circular in outline, and do not show such extreme prominences, and the air surfaces are sprinkled with golden brown granules and appear as faint lines in the photomicrograph.

We now turn to the appearance of the terminal bronchiolar epithelium of the mouse after one of the more usual methods of preparation (Fig. 12). This lung was not silverized, but was fixed in the same way as the others by injection of Bouin's fluid (Masson, 1929 (11)). The dark projections with rounded ends represent the light cells, and among them, suggesting gaps in a row of teeth, are low regions filled in by the dark cells, which now appear of lighter tone. One of these *d c* is distinctly of triangular contour, with an almost needlelike apex, which on higher magnification suggests the tip of a wetted and compacted camel's hair brush—but discrete cilia cannot be made out. Riding upon the marginal light cells are two dust cells *du c*. It seems probable that these are on their way from the alveoli to the region of the pharynx. Part of the epithelium shown in Fig. 12 appears under the oil immersion lens as in Fig. 13. The sharp point of the triangular dark cell *d c* might be called a stereocilium; but there is nothing here to decide the question of whether or not it is motile.

Fig. 14 is an oil immersion photomicrograph of the epithelium of a terminal bronchiole of the golden hamster. It is appended as evidence that the specializations already noted in the dark and light cells are not peculiar to the mouse. The short heavily silverized dark cells and the relatively opulent light cells are as sharply contrasted as those of the mouse.

Control lung sections, cleared without staining, from animals whose fresh lungs were fixed intrabronchially without silverization, showed no dark granules like those herein described for the silverized material.

Discussion

It is not intended here to deal with the epithelium of the terminal bronchioles in a broad way, but to highlight the peculiar and striking surface phenomenon attained by supravital washing with ammoniacal silver solution. Apparently the two types of cells of the terminal bronchioles have been known for almost (if not more than) 70 years; for Miller (1932 (12)) wrote (p. 134): "As stated by Kölliker (1881 [3]), ciliated epithelium extends beyond the point where goblet cells disappear; it does not, however, form a complete layer; for, interspersed with the ciliated cells, there are numerous non-ciliated cells." This statement Miller (1947 (13, p. 19)) repeated. Speaking of bronchiolar epithelium Miller (1932 (12, p. 137)) wrote: "In a longitudinal section of a bronchiolus which is dividing into bronchioli respiratorii a still further transformation of the epithelium can be followed. The cilia-bearing cells gradually disappear and are replaced by non-ciliated cuboidal epithelium which can be best seen in transverse sections of bronchioli respiratorii which have but few

alveoli along their walls." Miller (1932 (12, p. 134)) also wrote: "Moreover, the type of the cilia-bearing cells changes from a columnar to a cuboidal epithelium, which is the predominating type of epithelium in bronchioli between 0.3 mm. and 0.4 mm. in diameter," and repeated this statement (1947 (13, p. 19)). He did not, however, describe any pyramidal cells with contracted apices such as I show in my Fig. 9. He mentioned the "finely granular ends" of the ciliated cells (Miller, 1932 (12, p. 135)).

I feel that the supravital silverization method that I have herein described makes visible my "dark cells" (which fundamentally are ciliated epithelial cells, although the cilia seem to become rudimentary, and even sometimes invisible with ordinary means) farther down the air tract than is possible by usual methods. Although the cuticular plates of the pyramidal cells in the marginal region of the terminal bronchiolar epithelium become very narrow, and separate cilia arising from them have not always been discernible, yet the presence of cilia on all of them has not been ruled out, and the occurrence of an outward-bound fluid current set up by them here is possible. Lacking this the dust cells must make their way toward the pharynx by their amoeboid efforts alone. This air channel, rather than the lymphatic vessels of the lung, is regarded as their normal highway in leaving the lung (Macklin, 1938 (7)). The paired granules seen in the cuticle in lateral views are doubtless the "basal corpuscles" related to the roots of the cilia, referred to by histologists. It is possible that the dark cells are homologous distally with the epicytes (septal cells, niche cells, alveolar granular cells) of the alveolar walls (Macklin, 1938 (6), 1946 (8)). Both become superficially marked by a beautiful golden brown granulation after supravital washing with silver solution.

The light cells, with their free processes, are prominent objects in the sections. These projections suggest villi, so that the term "villus cells" is not inappropriate (Macklin, 1948 (10)). They are often shorter at the distal edge of the epithelium than a short distance proximal to this. If a fixative having a shrinking action is used, the sides of the processes are approximated, and then the presenting air surface looks fairly smooth, or at most gently scalloped. These villus processes are best seen by using the intrabronchial fixation method, with very low pressure. In ordinary immersion-fixed material, such as that used by pathologists, the processes are crowded together and hence seen with difficulty if at all. There may be an homology distally between the light cells and the so-called "bare areas" (Macklin, 1937 (5); 1938 (6)) of the alveolar walls, by some workers thought to be occupied by thin squames of epithelial origin, invisible in ordinary histological preparations. The possibility of dust and foam cells of the alveoli originating from the bronchiolar cells has been discussed (Macklin, 1947 (9)). The specialized nature of the mitochondria in the light cells (Macklin, 1948 (10)) is being presented in a separate paper.

From the foregoing exposition it is clear that the usual descriptions of the terminal bronchiolar epithelium are inadequate for the mouse. Most of the histological texts (as, for instance, Miller, 1932 (12) and Heiss, 1936 (1)) present the ciliated cells as here "disappearing" and being replaced by cuboidal cells, or as losing their cilia and becoming cuboidal. It would seem that the light cells herein described represent many at least of the "cuboidal epithelial cells" of these descriptions, though there is nothing to prove that they have ever borne cilia. It is possible that they may be homologous proximally with the goblet cells found in larger bronchioles. It has been herein noted that the findings in the golden hamster by this silverwash method are like those in the mouse. Sections from Bouin-fixed material, stained by common methods, have been examined in nine other mammals—rat, baboon, dog, goat, guinea-pig, monkey, rabbit, cat, man—and representatives of what are taken to be the two types of cells were found in the epithelium of the finest bronchioles. From these results it is felt that the pattern, morphology, and function of the dark and light cells are here basically comparable throughout this class, though there are notable species differences of detail.

Acknowledgments

Appreciation is expressed to the National Research Council of Canada for aiding this research project with a grant for technical assistance, and to Mr. Charles E. Jarvis for his excellent work in the preparation of the micro-sections and photomicrographs.

References

1. HEISS, R. Der Atmungsapparat. Handbuch der mikroskopischen Anatomie des Menschen. *Edited by* W. v. Möllendorff. Vol. 5, Part 3. Julius Springer, Berlin. 1936.
2. HODGMAN, C. D. and LANGE, N. A. Handbook of chemistry and physics. 15th ed. The Chemical Rubber Publishing Co., Cleveland, Ohio. The Norwood Press, Norwood, Mass. (The formula is from Miller's Laboratory Physics, Ginn & Co., Publishers, by permission). 1930.
3. KÖLLIKER, A. Zur Kenntnis des Baues der Lunge des Menschen. Verhandl. physik.-med. Ges. Würzburg, n. F. 16 : 1-24. 1881.
4. MACKLIN, CHARLES C. The musculature of the bronchi and lungs. Physiol. Revs. 9 : 1-60. 1929.
5. MACKLIN, CHARLES C. The "bare areas" outlined by silver deposit on the alveolar walls, as seen *en face* in thick flattened frozen sections of cat's lung. Trans. Roy. Soc. Can. V, Ser. 3, 31 : 63. 1937.
6. MACKLIN, CHARLES C. The silver lineation on the surface of the pulmonic alveolar walls of the mature cat, produced by applying weak silver nitrate solution and exposing to sunrays or photographic developer. J. Thoracic Surg. 7 : 536-551. 1938.
7. MACKLIN, CHARLES C. Evidence for the removal of airborne particulate matter from the lung alveoli *via* the bronchial tree. Trans. Roy. Soc. Can. V, Ser. 3, 32 : 149. 1938.
8. MACKLIN, CHARLES C. Residual epithelial cells on the pulmonary alveolar walls of mammals. Trans. Roy. Soc. Can. V, Ser. 3, 40 : 93-113. 1946.
9. MACKLIN, CHARLES C. The foam cells of mammalian lungs with special reference to the vacuoloids. Paper given at the Congressus VI Internationalis Cytologicus, Stockholm, Sweden. July, 1947. (Publication pending.) 1949.

10. MACKLIN, CHARLES C. Mitochondria of the villus epithelial cells of the finest bronchioles of the albino mouse, and their experimental alteration *in vivo* by ascorbic acid. Proceedings of the Canadian Physiological Society, Twelfth Annual Meeting, Laval University, Quebec City, Oct. 15th and 16th, 1948. Publication pending in the Revue canadienne de Biologie.

11. MASSON, P. Some histological methods: trichrome stainings and their preliminary technique. J. Tech. Methods and Bull. Intern. Assoc. Med. Museums, 12 : 75-90. 1929.

12. MILLER, W. S. The epithelium of the lower respiratory tract. Sec. V, Special cytology, pp. 133-150. 2nd ed. *Edited by* E. V. Cowdry. Paul B. Hoeber, Inc., New York. 1932.

13. MILLER, W. S. The lung. 2nd ed. Charles C. Thomas, Publisher. Springfield, Ill., U.S.A. 1947.

ANALYSIS OF THE ESSENTIAL STRUCTURAL FEATURES OF DDT BY A STUDY OF THE TOXICITY OF CLOSELY RELATED COMPOUNDS TO ROACHES AND TO HOUSEFLIES[1]

By Jean P. Picard[2] and C. W. Kearns[3]

Abstract

Three series of compounds have been prepared and compared for the insecticidal activities against German roaches and houseflies. The substituted 2,2-diphenyltrichloroethanes and substituted benzohydrols have been found to have comparable activities on roaches. The substituted 2,2-diphenyltrichloroethanes alone had insecticidal properties against houseflies. The benzophenone derivatives were ineffective against both groups of insects.

I. Introduction

Since the discovery of the outstanding insecticidal properties of DDT (1-trichloro-2,2-*bis*-(*p*-chlorophenyl)-ethane) in 1939, a considerable amount of work has been published on the relationship between the chemical structure and the activity of the DDT molecule as an insecticide.

Lauger *et al.* (1944 (6)) was probably the first to point out the possible relation between the chemical structure and the toxicity of DDT. He postulates that the extreme poisoning effect of DDT is due to the basic toxicity of *p*-bridged 4,4′-dichlorodiphenyl structure, which probably acts by an enzyme-blocking mechanism while the chloroform residue, he suggests, acts like a lipophylic group, which causes the molecule to have an affinity for the nerve lipoids.

On the other hand Fleck and Haller (1944 (4)), from a study of the ease with which hydrochloric acid could be released catalytically from the DDT molecule, have suggested that the insecticidal properties of DDT might be due to the catalytic decomposition by a naturally occurring catalyst. The same year Martin and Wain (1944 (7)) have reported that 4,4′-dichlorobenzophenone and 4,4′-diphenylacetic acid were almost inactive as contact insecticides and stomach poisons indicating that the $CHCCl_3$ group is associated with the toxic properties of the molecule. They claim that the hypothesis of the intracellular decomposition of DDT is preferred. The toxicity of the latter is ascribed to the hydrochloric acid simultaneously produced by either elimination or reduction. According to these workers, the insecticidal properties of a compound to which this hypothesis is applicable would depend on the ease with which hydrochloric acid is produced, provided that the compound is stable enough to survive translocation to its site of action.

[1] *Manuscript received September 1, 1948.*
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In view of these facts we thought that some information could be obtained about this subject by first preparing analogues of the DDT molecule varying the para-substituent and measuring quantitatively their relative potency, and then comparing measurements with similar molecules known to be incapable of dehydrohalogenation.

Analogues of the DDT molecule were synthesized and compared to closely related compounds in which the $CHCCl_3$ of the former was replaced by C=O and by CHOH. This last group of compounds has not been previously investigated for insecticidal properties.

The preparation of these compounds and the evaluation of their relative potency are the object of the present study.

II. Preparation of Compounds

Three series of compounds were prepared and evaluated on insects: Series 1, substituted 2,2-diphenyltrichloroethanes; Series 2, substituted benzophenones; Series 3, substituted benzohydrols.

1. Substituted 2,2-Diphenyltrichloroethanes

The syntheses of the DDT analogues was carried out by the Baeyer reaction (1827 (1)) between chloral and aryl derivatives using for the condensing agent a 1 to 1 mixture of concentrated sulphuric acid and fuming sulphuric containing as much as 20 to 30% of sulphur trioxide.

The condensation of chloral with monohalogenated benzene derivatives proceeds readily in presence of sulphuric acid mixture but in the case of the alkyl and alkoxyl benzene derivatives, owing to their high reactivity, a mixture of acetic acid and concentrated sulphuric acid was used instead of the usual condensing medium.

2. *Substituted Benzophenones*

The preparation of the substituted benzophenones was accomplished through the extension of a method used by Boesken (1905 (2)) for the preparation of benzophenone dichloride by the action of carbon tetrachloride on benzene in the presence of anhydrous aluminium chloride. When these compounds were subsequently hydrolyzed by steam, their corresponding ketones were obtained.

This method of preparation for symmetrical halogen and alkyl substituted ketones seemed more efficient than the process of condensing substituted benzoyl chloride with halogen or alkyl derivatives of benzene.

The condensation of monohalogenated and monoalkylated derivatives of benzene proceeded at room temperature without the formation of resinous by-products.

Attempts to prepare the 4,4′-dimethoxyl by the same method were unsuccessful.

3. Substituted Benzohydrols

The symmetrical aromatic ketones previously prepared were reduced to the corresponding secondary alcohol in almost quantitative yield by the use of aluminium isopropylate.

Since the proposal of this method by Meerwein and Schmidt (1925 (8)) and by Ponndorf (1926 (9)) for the reduction of a carbonyl group to the corresponding alcohol an extensive amount of work with this method has been reported, but as yet nothing has been published on the application of this method to the symmetrical substituted aromatic ketones.

The aromatic ketones can also be reduced to the corresponding secondary alcohol by alcoholic alkali and zinc dust. The only drawback to this method is the formation of pinacol, which, according to Cohen (1919 (3)), is the first step in the reduction.

III. Methods of Testing

1. Using Roaches

A quantitative measurement of the relative potency of each compound prepared was accomplished by determining the amount of the compound, in milligrams per 929 cm.^{2}* required to kill 50% adult male German roaches, *Blatella germanica* L., continuously exposed for 96 hr. to surfaces coated with the compound. This amount was called the LD_{50} (lethal dose to kill 50% for 96 hr. exposure).

The actual testing of the material was carried out by coating pint jars with acetone solutions containing various concentrations of the compound to be tested. The total surface area of a jar was found to be 929 cm.^{2}* Knowing the surface to be coated, the concentration of the solution to be used, and the number of mgm. per 929 cm.^{2} desired, the proper volume of the acetone solution was added to the jar by means of a 0.05 cc. graduated burette. The jars were uniformly coated by rotating them by hand until complete evaporation of the acetone solution. Ten male German roaches, after anesthesia with carbon dioxide, were confined in each jar and a minimum of 10 jars were prepared for each concentration to be tested.

2. Using Houseflies

The compounds prepared in this study have also been evaluated as contact insecticidal sprays on the housefly, *Musca domestica* L., by a wind tunnel method.

The apparatus used consists of a small wind tunnel, described by Roan and Kearns (1948 (10)). The air stream and the atomized spray droplets are pulled from one end to the other by means of an electrically driven blower. The test cage is placed in the apparatus in such a manner that it forms a section of the wind tunnel through which the spray flows. The time of exposure is limited by the velocity of the air stream and by the air pressure used to operate the atomizer.

* *929 cm.^{2} = 1 sq. ft.*

The flies, after being sprayed, are transferred to recovery cages. These cages consist of one quart cardboard liquid containers with the cardboard ends removed. The end and covers are replaced by 16 mesh galvanized screen of the same diameter. The container is lined with a section of kraft wrapping paper, which may be removed and discarded after use. The screen ends and test cages are decontaminated by standing over night in a solution of alcoholic potassium hydroxide.

The material to be tested is dissolved to the proper concentration in a highly refined kerosene ("Deo-base"). The volume of the spray solution is 0.5 cc. Each test was repeated five times. Checks run with 0.7 cc. of "Deo-base" alone gave less than 5% mortality.

IV. Results

The replacement of $CHCCl_3$ by CHOH in the 4,4′-disubstituted analogues of DDT, in which both substituents were fluorine, chlorine, bromine, methyl, and methoxyl, appear to be as toxic to male German roaches as the corresponding DDT analogue.

The same is true of the analogue without substituent in either ring although a considerable decrease in activity is apparent.

The LD_{50} for each compound was determined by plotting on logarithmic-probability paper, the percentage mortality at various concentrations. These results are shown in Tables I and II.

The benzophenone derivatives have been tested at 10 times the concentration required by the corresponding hydrol to kill 50% of the roaches, and even at that high concentration none of them show any sign of activity.

The results obtained for these same compounds tested as insecticidal sprays against houseflies are illustrated in Table III. In this experiment, if a compound did not show any activity at a concentration of 5 mgm. per cc. or 0.5%, it was not investigated further. This was the case with benzophenone and benzohydrol derivatives, which were found to be ineffective at a concentration of 5 mgm. per cc.

The 4,4′-dimethoxyl compound, although slightly less effective than DDT, has a considerable "knockdown" effect.

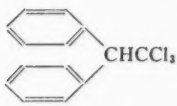
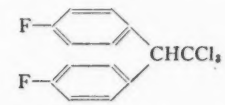
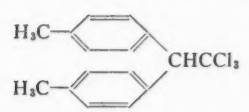
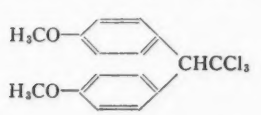
V. Discussion

In attempts to explain the essential structural features of DDT (1-trichloro-2,2-*bis*(*p*-chlorophenyl)-trichloroethane), several theories have been proposed.

The necessity of $CHCCl_3$ group as lipophylic agent postulated by Lauger *et al.* (1944 (6)), the possibility of dehydrohalogenation at the site of the action proposed by Martin and Wain (1944 (7)), the ease with which the DDT molecule could lose hydrogen chloride in presence of a naturally occurring catalyst, and the spatial relationship of the different parts of the molecule considered by Gunther and Metcalf (1946 (5)) are among the most important theories suggested for the mechanism of action of DDT-type compound.

TABLE I

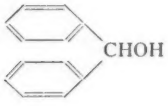
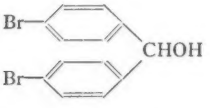
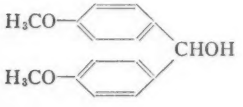
PERCENTAGE MORTALITY OF MALE GERMAN ROACHES EXPOSED TO SURFACES TREATED WITH VARIOUS AMOUNTS OF SUBSTITUTED 2,2-DIPHENYLTRICHLOROETHANE

Compound	Mgm./929 cm.²	% Mortality at 96 hr. exposure	LD_{50} *
	5 10 20 40 50	32 42 57 64 70	15
1-Trichloro-2,2-diphenylethane			
	0.01 0.05 0.1 0.4 1	42 56 65 77 82	0.025
1-Trichloro-2,2-*bis*-(*p*-fluorophenyl)-ethane			
	0.2 0.3 0.5 1. 1.5	4 11 20 43 60	1.15
1-Trichloro-2,2-*bis*-(*p*-chlorophenyl)-ethane			
	0.5 1 3 4 10	11 18 42 58 70	3.4
1-Trichloro-2,2-*bis*-(*p*-bromophenyl)-ethane			
	2 5 10 15 20	36 50 65 70 77	4.6
1-Trichloro-2,2-*bis*-(*p*-methylphenyl)-ethane			
	0.5 1 2 3 4	6 14 36 49 62	3.2
1-Trichloro-2,2-*bis*-(*p*-methoxyphenyl)-ethane			

* LD_{50} (*lethal dose for 50% mortality*) *in mgm./929 cm.² after 96 hr. exposure.*

TABLE II

PERCENTAGE MORTALITY OF MALE GERMAN ROACHES EXPOSED TO SURFACES TREATED WITH VARIOUS AMOUNTS OF SUBSTITUTED BENZOHYDROL

Compound	Mgm./929 cm.²	% Mortality at 96 hr. exposure	LD_{50}*
	5 10 20 40 60	34 43 54 63 72	15
Benzohydrol			
	0.01 0.05 0.1 0.5 1	40 52 60 66 72	0.03
4,4′-Difluorobenzohydrol			
	0.3 0.5 1 1.5 2	9 20 36 55 64	1.3
4,4′-Dichlorobenzohydrol			
	0.5 1 3 5 10	10 18 38 54 86	4
4,4′-Dibromobenzohydrol			
	1 2 5 10 20	24 35 53 67 80	4.2
4,4′-Dimethylbenzohydrol			
	0.5 1 2 3 5	4 15 31 50 61	3.5
4,4′-Dimethoxybenzohydrol			

* *LD_{50} (lethal dose for 50% mortality) in mgm./929 cm.² after 96 hr. exposure.*

TABLE III

PERCENTAGE MORTALITY, AFTER 24 HR., FOR HOUSEFLIES SPRAYED WITH 0.5 CC. OF VARIOUS CONCENTRATIONS OF SUBSTITUTES 2,2-DIPHENYLTRICHLOROETHANE

Compound	Mgm./cc.	% Mortality after 24 hr.	LD_{50}*
	5	0	
1-Trichloro-2,2-diphenylethane			
	2.5 2 1 0.5	95 86 17 50	0.55
1-Trichloro-2,2-*bis*-(*p*-fluorophenyl)-ethane			
	0.75 0.5 0.25 0.1	94 85 70 41	0.17
1-Trichloro-2,2-*bis*-(*p*-chlorophenyl)-ethane			
	1 0.75 0.5 0.25	95 82 70 50	0.28
1-Trichloro-2,2-*bis*-(*p*-bromophenyl)-ethane			
	5 4 2.5 1.5	94 84 60 22	2.20
1-Trichloro-2,2-*bis*-(*p*-methylphenyl)-ethane			
	2 1 0.75 0.5	94 70 40 29	0.80
1-Trichloro-2,2-*bis*-(*p*-methoxyphenyl)-ethane			

* LD_{50} *(lethal dose for 50% mortality) in mgm./cc. after 24 hr.*

The high activity of the hydrol corresponding to the DDT analogues, leads one to believe that the lipoid soluble group $CHCCl_3$ is not necessarily required in order for the DDT-type compound to be active and that a molecule of this type, deprived of its ability of losing hydrogen chloride, could still act as an insecticide.

In regard to insecticidal activities, the compound must be substituted in the 4,4′-positions, as demonstrated by the low activity of unsubstituted analogues, and the substituents must be of limited size. This point is well elucidated by the low LD_{50}'s found for the 4,4′-dimethoxyl and 4,4′-dimethyl compounds as compared with their next higher homologues. The difference is at least 10-fold.

The fact that toxicity in the DDT analogues vanished with the increase in the *p*-alkyl or *p*-alkoxyl substituents, indicates that enhanced paraffinic character is not beneficial even if it favors oil-solubility of the product.

The variation in toxicity within the 2,2-disubstituted ethanes, the difference in activity with the benzohydrol derivatives, and the inactivity of the benzophenones, when tested on two different groups of insects, suggest that the electronegative character of the substituents and the lipophylic action of the $CHCCl_3$ group cannot alone account for the significant action of this general type of structure, but rather the whole shape of the molecule must be considered. A mere mechanical view of tissue penetration by any contact poison leads to this consideration. This is also supported by the fact that molecules of other than a certain size were found to possess low activity. The nonrequirement for the molecule to bear a $CHCCl_3$ group also supports this view.

These findings tend to demonstrate the importance of exploring the potency of related compounds on more than one species of insects before postulating the relationship between the chemical structure and toxicity. This point is strikingly illustrated by the high activity of the hydrols on the German roach and their relative inactivity when applied to housefly.

VI. Acknowledgment

The authors are indebted to the Department of Lands and Forests of Quebec for the generous financial support of this project. Thanks are also due to Miss Amy Kell and Mr. H. K. Miller for aid in the experimental work.

References

1. BAEYER, A. Uber die Verbindungen de Aldehyd mit den Phenolen und aromatischen Kohlenwasserstoffen. Ber. 5 : 1094-1100. 1827.
2. BOESKEN, M. I. La formation de dichlorure de benzophenone par l'action du tetrachlorure de carbon sur le benzene. Rec. trav. chim. 24 : 3-5. 1905.
3. COHEN, M. D. La reduction des cetones aromatiques (suite). Rec. trav. chim. 38 : 113-131. 1919.
4. FLECK, E. E. and HALLER, H. L. Catalytic removal of hydrogen chloride from some substituted alpha-trichloroethanes. J. Am. Chem. Soc. 66 : 2095. 1944.

5. GUNTHER, F. A. and METCALF, R. L. Mechanism of insecticidal action. Derivatives of chloro- and fluoro-diphenyl ethane. Am. Chem. Soc. Abstracts Papers, 110th meeting, Chicago. p. 40B. 1946.
6. LAUGER, P., MARTIN, H., and MULLER, P. Uber Konstitution und toxische Wirkung von naturlichen und neuen synthetischen insektentonden Stoffen. Helv. Chim. Acta, 27 : 892-928. 1944.
7. MARTIN, H. and WAIN, R. L. Insecticidal action of DDT. Nature, 154 : 512-513. 1944.
8. MEERWEIN, H. and SCHMIDT, R. Ein neues Verfuhren zur Reduction von Aldehyden und Ketonen. Ann. Chem. Justus Liebigs, 444 : 221-238. 1925.
9. PONNDORF, W. Der reversible Austausch der Oxydationsstufen zwischen Aldehyden oder Ketonen einerseits und primaren oder sekundaren Alkoholen anderseits. Z. angew. Chem. 39 : 138-146. 1926.
10. ROAN, C. C. and KEARNS, C. W. Testing insecticidal sprays. Soap Sanit. Chemicals, 24 : 133-150. 1948.

THE FUR RESOURCES OF CHAPLEAU DISTRICT, ONTARIO[1]

By Randolph L. Peterson[2] and Vincent Crichton[3]

Abstract

Records of the area trapped and of legally caught furs in the Chapleau district, Ontario, have been made for the seasons 1941-42 to 1946-47. From these records the area required to produce one harvested animal has been computed for most species, which should be of considerable value to persons concerned with fur production in Canada. These figures should also be useful to students of animal populations since they take into account, at least in part (specific areas actually trapped), the variation in trapping pressure that cannot be reflected in figures based on total numbers taken from large areas. This six year period seems sufficient to indicate significant trends in populations, which in certain cases might be predicted by study of past trends of province-wide returns.

During the six year period the average return value for each square mile trapped was $16.90, which netted each trapper using one township an average of $607.00 each year. The average total annual fur resources amounted to $74,000.00.

The distribution of the catch of marten and fisher in the district suggests that the Chapleau Crown Game Preserve may be influencing the catch of these two species in nearby territories.

Introduction

The value of wild caught furs of Canada's 31 million dollar fur resources amounted to approximately eighteen and one-half million dollars in the season 1944-45 (1). Although total fur return figures are available for the various Provinces and for the Dominion as a whole, these figures give no index to specific fur production from small comparable areas under various levels of trapping pressure. It appears desirable to know what furs have been taken from a specific area and what relation the trapping pressure has to the size of the area necessary to produce one trapped animal. Even within a district the size of Chapleau, the actual area trapped varies from year to year. In other words, how large an area is necessary to produce one harvested animal unit for each of the various species being taken from any given district?

The Chapleau Forest District (Fig. 1) is an administrative unit of the Ontario Department of Lands and Forests (formerly under the Game and Fisheries Department and later amalgamated with the Department of Lands and Forests as the Fish and Wildlife Division). Coauthor Crichton, an officer of the Fish and Wildlife Division of the Department, has the responsibility of administering the fish and wildlife resources of this district. His primary responsibility is law enforcement, with some biological investigations as well as administration. The area has been divided into trapping grounds

[1] *Manuscript received in original form May 4, 1948, and, as revised, November 13, 1948. Contribution from the Division of Mammalogy, Royal Ontario Museum of Zoology, and the Fish and Wildlife Division, Ontario Department of Lands and Forests, Toronto, Ont.*

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and each trapper assigned to a definite trapping area. For the most part each trapping ground is equivalent to one township (36 square miles), although some trappers have been allotted one and one-half or two townships. These

FIG. 1. *Map of part of the Province of Ontario showing the Chapleau Forest District and the Chapleau Crown Game Preserve.*

grounds are usually held year after year by the same trapper (Fig. 2). Each trapper is required to report the exact number of furs of each species taken. (The illegal trapping presents a factor that cannot be properly evaluated here.) With the exception of beaver, there was no restriction on the number of pelts that could be taken. Over the period here discussed, the take of beaver was regulated by the Provincial Government to insure sustained annual yield and was limited roughly to an average of 10 beaver per trapping ground. Beginning with the season 1941-42, a careful record of each pelt legally taken from each ground has been made under the supervision of coauthor Crichton. The annual catch from each individual trapping ground has been compiled and these data computed for the entire area trapped in the Chapleau district, with the exception of the southernmost 14 townships, which were added to the district beginning with the 1946-47 season. For purposes of this paper these townships are considered as not being trapped and are not included with the area trapped except in the 1946-47 season. Thus, prior to 1946-47, the actual production of the entire district was greater than the figures here given but the size of the unit area required to produce each pelt was not affected.

The relative intensity of trapping pressure within the various trapping grounds cannot be properly evaluated or compensated for in computing the size area necessary to produce one animal unit because of differences in

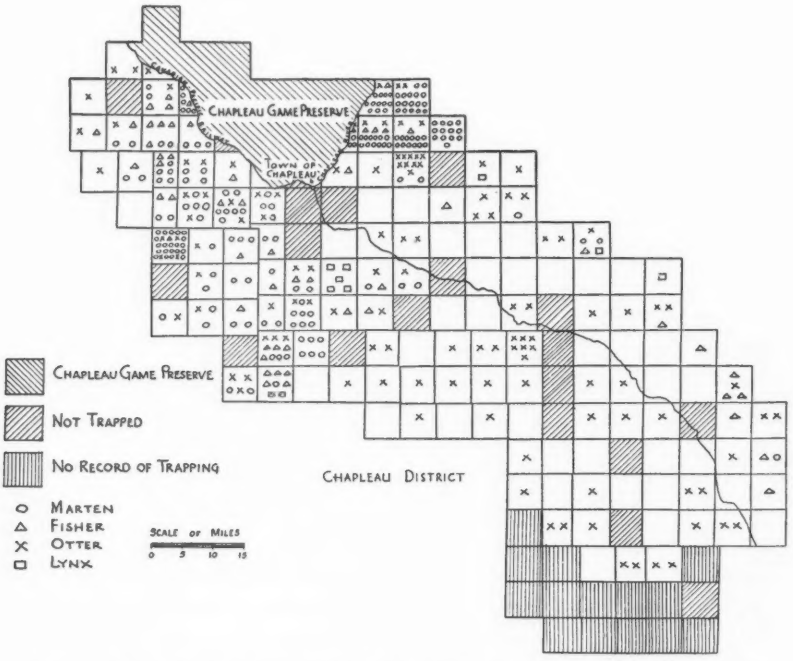

FIG. 2. *Map of the Chapleau Forest District showing area trapped in 1945-46 and distribution of catch of marten, fisher, otter, and lynx for that season.*

trapping techniques, number of traps utilized, general skill and ability of trappers, intensity and length of active trapping, amount of selective trapping, and various other uncontrollable factors. Further, it should be remembered that several types of habitat are found throughout the district, thus the productivity for the various species necessarily varies even within small localities. A notable example is the habitat of the muskrat; its aquatic habitat occurs only in certain townships and in those is often restricted to a small percentage of the area. On the other hand, most of these trapping grounds are trapped annually by the same trappers, which should tend to create fairly equal harvesting pressure except in cases where changes in relative value of various species and other factors have tended to cause selective trapping for the more valuable types. Even so, it still seems highly desirable to know what the average productivity of a given tract of northern Ontario land might reasonably be expected to be under more or less "normal" trapping pressure.

Although comparable figures for fur production of other districts within Ontario are not available, the Chapleau district probably rates well above average in fur production for areas of comparable size throughout the Province.

We are grateful to Prof. J. R. Dymond, Director, Royal Ontario Museum of Zoology; Dr. C. H. D. Clarke, Wildlife Supervisor, Ontario Department of Lands and Forests; Dr. Walter P. Taylor, Senior Biologist, United States Fish and Wildlife Service; and others for their co-operation, suggestions, and reading of the manuscript.

Description of Area

The Chapleau Forest District, lying east of Lake Superior, averages roughly 60 miles wide and extends some 190 miles northwest and southeast along the Canadian Pacific Railway, which bisects the area lengthwise (Fig. 1). The total area is approximately 6384 square miles, about 600 square miles of which lies within the Chapleau Game Preserve, which extends into the northern portion of the forest district, leaving roughly 5780 square miles of trapping grounds.

The topography is made up of many lakes and streams winding through low rolling ridges with considerable outcroppings of rocks, especially along and south of the height of land that extends across the northern portion of the district. The geologic formations are chiefly Precambrian, with most of the district lying on the Canadian Shield. The town of Chapleau, situated just north of the height of land, has an elevation of 1412 ft. above sea level. The district is divided into three watersheds. The drainage of the northern portion flows into the Moose River drainage to James Bay. The northwestern portion lies in the Lake Superior drainage, while the remaining southern portion is situated in the Lake Huron drainage.

The entire Chapleau district would fall within the Canadian Life Zone of Merriam, Bailey, Nelson, and Preble (13) but certainly there are marked differences between the flora of the northern and southern portions of the district. Several species of trees reach their northern limits in the Chapleau vicinity. These include white pine, hemlock, yellow birch, red maple, sugar maple, striped maple, basswood, red oak, black cherry, beech, and others. This would seem to indicate a valid separation of biotic areas within this area as proposed by Halliday (8) and Dice (7). Jack pine is the only pine found to any extent in the far northern portion of Chapleau district, while both white and red pine occur with it in the southern portions. The common species found throughout the district include black spruce, white spruce, white birch, white cedar, quaking aspen, balsam poplar, large-toothed aspen, cherries, Juneberry, and several smaller shrubs such as alders, dogwood, and willows. The southeastern half of the district has been extensively logged and burned, resulting in large areas of second growth poplar and white birch on rather thin soils.

For a list of the summer birds for the Chapleau area see Baillie and Hope (2).

History of the Area

During the early exploration and trading in northern Canada the Hudson's Bay Company restricted most of its posts and settlements to the coastal areas. One of their earliest inland posts was a temporary one called Brunswick House built about 150 miles north of the Chapleau area on the Missanabie River in 1776 (10). According to Voorhis (14) this post was abandoned in 1790 and a New Brunswick House was substituted. The latter was built in 1778 at the north end of Brunswick Lake, which is now a part of the Chapleau game preserve. Later several other Hudson's Bay posts were established in this general area, which included the Chapleau Post, Biscotasing Post, Flying Post, Missanabie Post, and Peterbell Post. With the exception of the last two, these posts were subsequently abandoned by the Company. "The North West Company also built a post on Missinaibi lake in 1800" (14).

At the close of the century the country was traversed by railroads and the forest began to be opened up by extensive logging and burning. It is reported that many extensive fires were deliberately set by the invading white men. Undoubtedly this change in forest composition has had its effect on the indigenous furbearing mammals. Our present day knowledge of the ecology of many of our native furbearers is so sketchy that it is extremely difficult to evaluate the effects of changes that have taken place in their environment.

Account of Furbearing Species

Order RODENTIA

Castor canadensis—Beaver

The beaver production in Chapleau district has fared well under a regulated harvest (Table I). The steady increase beginning in 1942-43 seems to indicate clearly the value of such a system for producing a sustained annual yield.

TABLE I

THE ANNUAL HARVEST OF BEAVER IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of beaver	1067	798	840	955	1081	1571	1052
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	4.1	4.6	4.7	4.5	4.2	3.5	4.2
Av. value per pelt, $	24.00	26.00	34.00	38.00	56.00	30.00	35.00
Total revenue, $	25,600.00	20,700.00	28,600.00	36,300.00	60,500.00	47,100.00	36,500.00
Av. return value per sq. mi. trapped	5.91	5.67	7.24	8.47	13.22	8.47	8.30

The area necessary to produce one harvested beaver varied only slightly for the period. On the average a little over four square miles of Chapleau country, if properly managed, will produce one harvestable beaver. Perhaps it may be possible to increase the number of animals that may be taken from certain areas, thus reducing the size area required to produce each individual; however, carefully regulated control should be exercised in such an attempt.

Ondatra zibethica—Muskrat

Although the number of muskrats produced in Chapleau district has been relatively small compared to other areas containing more extensive favorable marshes, the harvest of the entire district for the past six years appears to be a fairly direct reflection of fluctuations in the total populations. The size area required to produce one harvested muskrat would seem to be a better reflection of the size of the actual population than the total number taken from the district because of the variation in the amount of land actually trapped (Fig. 3). The fluctuation in numbers has not been violent since each trapper

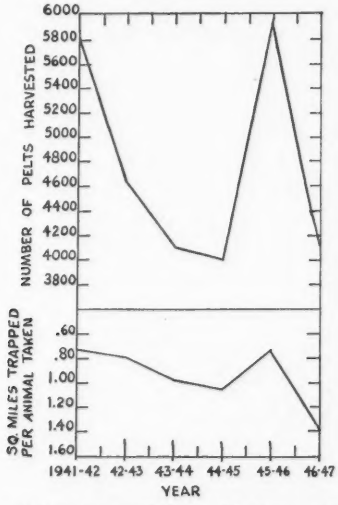

FIG. 3. *Top. Total muskrat produced from Chapleau district. Bottom. Area (in square miles) trapped to produce one animal unit.*

took, on the average, between 25 and 46 animals annually with an average of 37 pelts (Table II); however, a general downward trend is suggested, which could be forecast by the study of past fluctuations in Provincial production (1).

Order CARNIVORA

Canis latrans—Brush Wolf, Coyote

Brush wolves seem to have entered the Chapleau district rather recently. According to Cross (4) they probably came from the west between 1900 and 1936. They have never become abundant in the area and only recently

TABLE II

THE ANNUAL HARVEST OF MUSKRAT IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of muskrat	5857	4613	4122	4009	5906	4118	4771
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	0.74	0.79	0.98	1.07	0.77	1.35	0.92
Av. value per pelt, $	2.00	2.00	2.25	2.75	3.40	2.00	2.45
Total revenue, $	11,700.00	9200.00	9300.00	11,100.00	20,100.00	8200.00	11,600.00
Av. return value per sq. mi. trapped	2.70	2.52	2.35	2.60	4.38	1.48	2.65

have we been able to obtain valid records of their occurrence substantiated by specimens. On Feb. 21, 1947, one specimen was killed by a train in Delmadge Township and turned over to the Royal Ontario Museum of Zoology. Since then several additional specimens have been obtained from the Chapleau district. There is little value in their pelt on current markets.

Canis lupus—Timber Wolf

Timber wolves are not uncommon in the Chapleau district. They do not constitute a noteworthy fur resource since there is a very poor market for this type of fur in Ontario.

Vulpes fulva—Red, Cross, and Silver Fox

The number of foxes has been increasing across Canada and at the same time showing cyclic fluctuations (3, 5). The harvest from Chapleau is given by color phases in Table III. As shown by Cross (6) and Butler (3), the color ratios vary from one geographical area to another with fairly consistent reduction in the percentage of occurrence of silver and cross phases in recent years. When comparing the color ratios of foxes of the Chapleau district with those of the two nearest Hudson's Bay Company posts listed by Cross (6) (Table IV), closer agreement is found with ratios from Gogama to the east than from Missanabie to the northwest.

Ursus americanus—Black Bear

Bear in the Chapleau district increased until 1945, then apparently decreased. The entire take of bear reported for 1944-45 in all of Ontario amounted to only 333 skins (1). Since the average values of these pelts amounted to only three dollars it is quite understandable why few are skinned for pelts. No record of sale of bear skins was made for the Chapleau area during 1941-47.

TABLE III

THE ANNUAL HARVEST OF FOX IN THE CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of red fox	198	315	333	304	389	375	319
Total catch of cross fox	36	36	21	32	34	24	31
Total catch of silver fox	—	4	1	6	5	1	3
Total catch all types fox	234	355	355	342	438	400	352
Sq. mi. trapped per animal unit harvested	18.5	10.3	11.1	12.5	10.7	13.9	12.5
Av. value per pelt red fox	5.00	6.00	6.25	7.25	8.75	4.00	6.30
Av. value per pelt cross fox	17.00	15.00	20.00	15.00	17.00	13.00	16.00
Av. value per pelt silver fox	18.00	18.00	18.00	18.00	25.00	18.00	20.00
Total revenue (all foxes)	1600.00	2500.00	2500.00	2800.00	4100.00	1800.00	2550.00
Av. return value per sq. mi. trapped (all foxes)	0.37	0.69	0.64	0.65	0.90	0.33	0.58

TABLE IV

COMPARATIVE RATIOS OF COLOR PHASES IN RED FOX (*Vulpes fulva*)

Area	Period	Red fox	Cross fox	Silver fox	Bastard fox
Chapleau district	1941-46	90.5	8.6	0.8	—
Gogama post	1916-20	91.3	8.6	—	—
	1934-38	92.9	5.7	0.5	0.7
	1916-35	86.6	10.1	3.0	0.1
Missanabie post	1916-20	81.1	16.2	2.5	—
	1934-38	84.7	12.3	0.7	2.1
	1916-35	83.4	13.7	2.0	0.7
Ontario	1916-20	71.1	23.9	4.4	0.4
	1934-38	78.0	18.7	2.2	0.9

Procyon lotor—Raccoon

The raccoon is a rare animal as far north as the Chapleau district. None was sold on the market from this area, although one was shot along the Wakami River in Benton Township in November, 1946, by a resident of Chapleau, and examined by us. It was reportedly mistaken for a "young wolf" and the pelt turned in to the Chapleau district office.

Mustela erminea—Ermine, Weasel

The Chapleau production of ermine (Table V) is comparatively small. The area involved in producing one harvested animal unit in this case probably has little significance since the average value of the pelts is so low. The trapping pressure for this species is only sporadic for the main efforts are directed toward more valuable furs.

TABLE V

THE ANNUAL HARVEST OF ERMINE IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of ermine	234	294	500	531	631	593	464
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	18.5	12.4	7.9	8.0	7.2	9.4	9.5
Av. value per pelt, $	0.50	0.55	0.80	0.90	1.30	1.30	1.00
Total revenue, $	117.00	162.00	400.00	478.00	820.00	771.00	458.00
Av. return value per sq. mi. trapped, $	0.03	0.04	0.10	0.11	0.18	0.14	0.10

Mustela vison—Mink

The production of mink has been fairly constant in the Chapleau area in recent years. A low for the period was the first year, 1941-42, when only 555 pelts were taken at the rate of one animal for each 7.8 square miles trapped. This was followed the second year, 1942-43, by a high for the period when 770 pelts were taken at the rate of one animal for each 4.7 square miles trapped. The rate for the remaining four years (Table VI) remained near the six year average of one animal for each 6.8 square miles trapped.

Martes americana—Marten

The trend in production of marten has been most encouraging in the Chapleau district (Table VII). There was almost a steady increase in numbers taken during the six year period 1941-42 to 1946-47 from a low of 58 to a high of 241. The area required to produce each individual (Fig. 4) is only a partial indication of the relationship of trapping pressure to the total numbers taken. The increasing in value per pelt undoubtedly affected a selective trapping pressure upon this species. It is of considerable interest to examine the distribution of the catch of marten as shown in Fig. 2 for the season 1945-46. If a line is drawn across the district dividing equally the trapping areas of the southeastern portion from the northwestern portion, it will be found that 98% of the marten are taken in that half of the district

TABLE VI

THE ANNUAL HARVEST OF MINK IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of mink	555	770	613	591	603	750	647
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	7.8	4.7	6.4	7.2	7.6	7.4	6.8
Av. value per pelt, $	12.00	11.00	18.00	20.00	33.00	22.00	19.00
Total revenue, $	6660.00	8470.00	10,900.00	11,800.00	19,900.00	16,500.00	12,400.00
Av. return value per sq. mi. trapped	1.54	2.32	2.77	2.76	4.35	2.97	2.82

TABLE VII

THE ANNUAL HARVEST OF MARTEN IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of marten	58	84	91	88	185	241	124
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	75	43	43	49	23	23	35
Av. value per pelt, $	33.00	30.00	39.00	42.00	50.00	40.00	41.00
Total revenue, $	1910.00	2520.00	3550.00	3700.00	9250.00	9640.00	5100.00
Av. return value per sq. mi. trapped, $	0.44	0.69	0.90	0.86	2.01	1.73	1.16

nearest the Chapleau Game Preserve. The northwestern portion contains more extensive stands of mature conifers, which presumably creates a more favorable habitat for this species, but in spite of these ecological differences there is strong evidence that some animals are emigrating from the preserve to surrounding territory. If emigration is not a major factor, and these animals are being produced in the area where taken, the actual area required to produce one harvestable marten would be reduced 50% for northern Chapleau district. Seemingly the game preserve possesses value as a reservoir for such furbearers.

On the whole the decrease in marten since 1919 has been alarming. In recent years the provincial output of marten has decreased over 75% from the 1919-20 level (1). If overtrapping has been one of the primary causes of reduction, it may be possible that the registered trapping ground system, which

has recently been inaugurated throughout Ontario, will forestall further decrease. If the trend of increase in the Chapleau remains a real one, certainly its trapping system could be cited as a favorable factor.

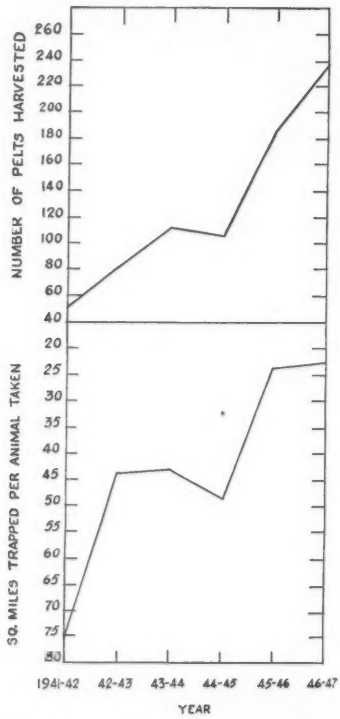

FIG. 4. *Top. Total marten production of Chapleau district. Bottom. Area (in square miles) trapped to produce one animal unit.*

Martes pennanti—Fisher

Like marten, the fisher showed an encouraging increase (Table VIII) from 1941-42 to 1946-47. Again the area required to produce one trapped animal should give a check on relative increase (Fig. 5). If, however, the district is divided, as suggested for the case of marten, 84% of all fisher caught in 1945-46 would be taken from the northwestern half (Fig. 2). As with the marten this suggests an overflowing effect from the game preserve.

The recent Ontario and Dominion production of fisher has been greatly reduced over past high years (1). Whether or not the trend of increase as shown by the Chapleau district will be reflected throughout the Province remains to be seen. Compared with figures for Ontario totals available for the same years, figures for the Chapleau district show that it is undoubtedly one of the better fisher producing areas of the Province.

TABLE VIII

THE ANNUAL HARVEST OF FISHER IN THE CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of fisher	20	17	22	45	56	92	42
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	216.0	215.0	179.0	95.0	82.0	60.0	104.0
Av. value per pelt, $	53.00	45.00	68.00	70.00	63.00	53.00	59.00
Total revenue, $	1060.00	765.00	1500.00	3150.00	3530.00	4880.00	2480.00
Av. return value per sq. mi. trapped, $	0.24	0.21	0.38	0.74	0.77	0.88	0.56

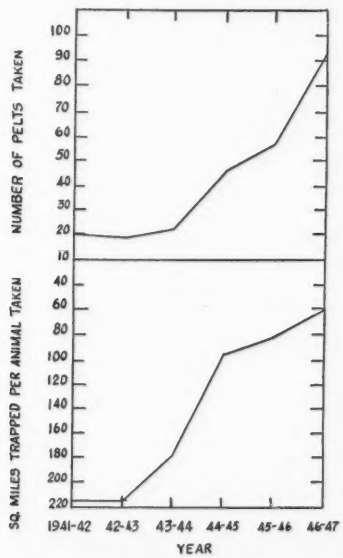

FIG. 5. *Top. Total fisher produced in Chapleau district. Bottom. Area (in square miles) trapped to produce one animal unit.*

Gulo luscus—Wolverine

According to the older settlers the last record of wolverines in Chapleau district was between 1885 and 1900. Wolverine were probably never very abundant in Ontario. The remaining few animals are restricted to the wilder portions of the far northwestern part of the Province. It would appear that the wolverine is doomed to extinction in Ontario. Since 1928-29 the annual total take has been less than 10 animals in all Ontario (1).

Lutra canadensis—Otter

The total number of otter taken from the Chapleau district increased steadily from 1941-42 to 1946-47 (Table IX). However, the size of unit area trapped to produce each pelt indicates some fluctuations in populations

TABLE IX

THE ANNUAL HARVEST OF OTTER IN CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of otter	63	66	68	70	125	131	87
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	69	55	58	61	37	42	50
Av. value per pelt, $	20.00	17.00	25.00	28.00	30.00	35.00	26.00
Total revenue, $	1260.00	1120.00	1700.00	1960.00	3750.00	4580.00	2400.00
Av. return value per sq. mi trapped, $	0.29	0.31	0.43	0.46	0.82	0.82	0.55

(Fig. 6). The distribution of catch for 1945-46 (Fig. 2) indicates a fairly even population throughout the district. The provincial catch of otter has remained fairly constant since 1919-20 (1), which apparently indicates that the otter is holding up well under existing trapping pressures.

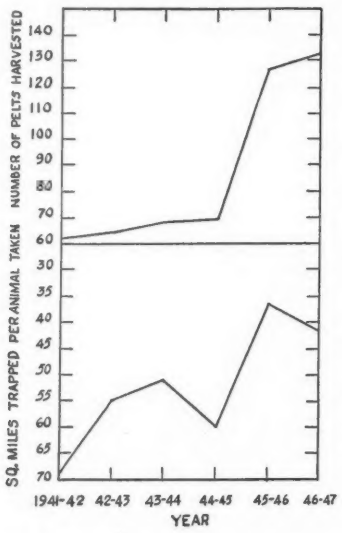

FIG. 6. *Top. Total otter produced in Chapleau district. Bottom. Area (in square miles) trapped to produce one animal unit.*

Mephitis mephitis—Striped Skunk

The recorded number of pelts taken from the Chapleau district gives no indication of the relative size of populations nor trends of abundance. The low price, coupled with the disagreeable task of handling, brings a very small fraction of available skunk pelts to the market. The average annual catch from 1941-42 to 1946-47 was only 19 skunks.

Lynx canadensis—Canada Lynx

The catch of lynx in the Chapleau district has been a small and variable one in recent years (Table X). MacLulich (11) plotted the lynx returns for Canada from 1751 to 1932. He found violent fluctuations in numbers trapped

TABLE X

THE ANNUAL HARVEST OF LYNX IN THE CHAPLEAU DISTRICT

	1941-42	1942-43	1943-44	1944-45	1945-46	1946-47	Average
Total catch of lynx	8	6	14	15	8	32	14
Total number of sq. mi. trapped	4332	3657	3946	4285	4580	5563	4394
Sq. mi. trapped per animal unit harvested	541	609	282	286	572	174	317
Av. value per pelt, $	37.00	40.00	50.00	47.00	50.00	42.00	44.00
Total revenue, $	296.00	240.00	700.00	705.00	400.00	1340.00	614.00
Av. return value per sq. mi. trapped, $	0.07	0.07	0.18	0.16	0.09	0.24	0.14

and demonstrated a cycle with peaks of abundance occurring on the average of every 9.7 years. He also (12) demonstrated a close correlation of the abundance of lynx with that of the varying hare (*Lepus americanus*). The number of lynx taken in the Chapleau district is so small (Fig. 2 and Table X) and the unit area necessary to produce one animal unit so variable that it is doubtful that the graphic indications for only six years can indicate any significant trend in productivity; however, a general increase seems evident, an increase that could be forecast by examination of provincial trends (1). The figures (Fig. 7) do indicate the erratic nature of lynx populations within a small area.

Lynx rufus—Bobcat

Although we have no specimens from the Chapleau district, we have obtained reports of four bobcats taken since 1919. Mr. Sam Chappise reported killing one in the district in 1919; Mr. Wilfred McAuley of Chapleau,

one in Lackner Township shortly afterward; Mr. Ignace Mamyguess of Chapleau, one in 9H Township in 1936; and Mr. Alec Bain, one in Rollo Township in 1942. Bobcats are quite rare as far north as Chapleau. However,

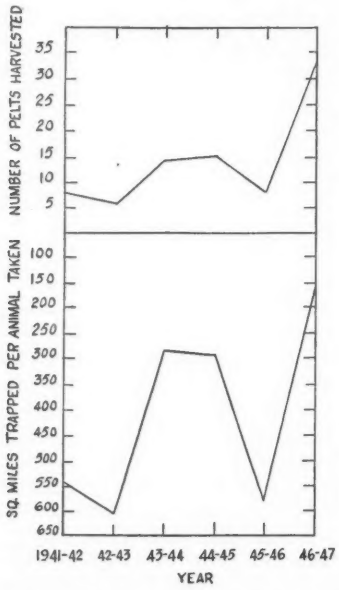

FIG. 7. *Top. Total lynx production of Chapleau district. Bottom. Area (in square miles) trapped to produce one animal unit.*

one pelt was received at the Hudson's Bay Post at Pagwa River in 1946 and presented to the Royal Ontario Museum of Zoology by Mr. W. Glennie, Manager of the post. This animal was a female, taken Nov. 2, 20 miles east of Pagwa River Post, some 40 miles north of the township of Rogers. This area lies 200 miles north of Chapleau. Mr. Glennie reports that to his knowledge no Indian or white trapper had ever heard of a bobcat in that area before.

Discussion

Two common species, the snowshoe rabbit or varying hare (*Lepus americanus*) and red squirrel (*Tamiasciurus hudsonicus*) do not constitute important fur resources in the area discussed, although an unknown number are taken by trappers each year. Hess (9), however, included the number of these species taken on an 89½ mile trap line over a 13 year period 1931-44 in the Oba-Hearst area, which lies some 100 miles north of Chapleau. Since the Province of Ontario requires no royalty on these species it is difficult to appraise the present or potential values of these two mammals.

A comparative summary of production of the principle furbearers of the Chapleau district is given in Table XI. It will be seen that beaver, muskrat, and mink constitute the more important species of the area in both numbers taken and in total value.

TABLE XI

COMPARATIVE SUMMARY OF PRODUCTION OF THE PRINCIPAL FURBEARERS OF CHAPLEAU DISTRICT

Species	Sq. mi. trapped per animal harvested			Catch per trapper (one twp.)			Return value, $, per sq. mi. trapped			Av. total revenue,	Av. total catch
	Av. low	Av. high	Six yr. av.	Av. low	Av. high	Six yr. av.	Av. low	Av. high	Six yr. av.	Six yr. av.	Six yr. av.
Beaver	3.5	4.7	4.2	7.2	9.6	8.2	5.67	13.22	8.30	36,500.00	1052
Muskrat	0.7	1.3	0.9	25.3	46.8	37.2	1.48	4.38	2.65	11,600.00	4771
Red fox	11.6	21.9	13.7	1.6	2.9	2.5	0.23	0.74	0.46	2000.00	319
Cross fox	101.0	232.0	144.0	0.15	0.33	0.24	0.06	0.15	0.11	490.00	31
Silver fox	—	5563.0	1550.0	—	0.05	0.02	—	0.03	0.01	57.00	3
Ermine	7.2	18.5	9.5	1.9	4.7	3.6	0.03	0.18	0.10	458.00	464
Mink	4.7	7.8	6.8	4.4	7.1	5.0	1.54	4.35	2.82	12,400.00	647
Marten	23.0	75.0	35.0	0.5	1.5	1.0	0.44	2.01	1.16	5100.00	124
Fisher	60.0	216.0	104.0	0.16	0.56	0.3	0.21	0.88	0.56	2480.00	42
Otter	37.0	69.0	50.0	0.5	0.9	0.7	0.29	0.82	0.55	2400.00	87
Lynx	174.0	609.0	317.0	0.05	0.16	0.1	0.07	0.24	0.14	614.00	14

Consideration of areas of comparative size required to produce each harvested animal unit should be useful in connection with future conservation and management of our furbearers and might well be the basis for further research.

Since trapping returns form a substantial part of the total income of many residents in northern Ontario, it is important to maintain the fur resources in continued production. The decrease in the number of certain of the fur-bearing mammals threatens one of Canada's most important heritages. Now is the time to follow up the important conservation policies already adopted by further measures based on both intensive and extensive research so that the fur production of Ontario and Canada may be maintained on the highest possible level.

References

1. ANONYMOUS. Fur production of Canada. Dept. Trade Commerce. Annual reports for years 1919-20 through 1944-45. Ottawa.
2. BAILLIE, JAMES L., JR. and HOPE, CLIFFORD E. The summer birds of Sudbury District, Ontario. Contribs. Roy. Ont. Museum Zool. 28 : 1-32. 1947.
3. BUTLER, L. Distribution and genetics of the colour phases of the red fox in Canada. Genetics, 30 : 39-50. 1945.
4. CROSS, E. C. Wolf! Wolf! Rod and Gun in Canada. Jan.: 18-19, 32-33. 1938.
5. CROSS, E. C. Periodic fluctuations in numbers of the red fox in Ontario. J. Mammal. 21 (3) : 294-306. 1940.
6. CROSS, E. C. Colour phases of the red fox (*Vulpes fulva*) in Ontario. J. Mammal. 22 (1) : 25-39. 1941.

7. DICE, LEE R. The Canadian biotic province with special reference to the mammals. Ecology, 19 (4) : 503-514. 1938.

8. HALLIDAY, W. E. D. A forest classification for Canada. Can. Dept. Mines Resources. Dominion Forest Service, Bull. 89 : 1-50. 1937.

9. HESS, Q. F. A trapper's record of animal abundance in the Oba-Hearst area of Ontario for the years 1931-1944. Can. Field-Naturalist, 60 (2) : 31-33. 1946.

10. MACKAY, D. The Honourable Company, a history of the Hudson's Bay Company. Bobbs-Merrill Company, N.Y. 1936.

11. MACLULICH, D. A. Sunspots and abundance of animals. J. Roy. Astron. Soc. Can. 30 : 233-246. 1936.

12. MACLULICH, D. A. Fluctuations in the number of varying hare (*Lepus americanus*). Univ. Toronto Studies, Biol. Ser. No. 43 : 1-136. 1937.

13. MERRIAM, C. H., BAILEY, V., NELSON, E. W., and PREBLE, E. A. U.S. Biological Survey fourth provisional zone map of North America. 1910. *From* Field Book of North American Mammals, *by* H. E. Anthony. G. P. Putnam's Sons, N.Y. 1928.

14. VOORHIS, ERNEST. Historic forts and trading posts of the French regime and of the English fur trading companies. Dept. Interior, Ottawa. 1930. (Mimeographed).

THE EFFECT OF DIETARY FAT ON THE HEAT TOLERANCE OF GOLDFISH (*CARASSIUS AURATUS*)[1]

By W. S. Hoar[2] and J. E. C. Dorchester[3]

Abstract

Three groups of goldfish were fed diets containing 25% fat. The diets for the three groups differed in the degree of unsaturation of these fats. Pilchard oil (iodine value 181.7), herring oil (iodine value 128.4), and lard (iodine value 66.2) were the fats used. Heat tolerance of the fish was then tested by holding them at a constant high temperature and observing the time of death. Variations in the ability of the groups to withstand high temperature were correlated with differences in the degree of unsaturation of their extracted fats. The iodine value of the herring oil diet was very close to that of the fats of the goldfish prior to feeding and no change in the value for fish fed on this diet was observed. Each of the other two diets changed the degree of unsaturation of the goldfish fats by approximately 54% of the maximum theoretical change. These changes modified the heat resistance of the fish. The degree of modification of heat tolerance, however, cannot be strictly correlated with the degree of change of unsaturation of fats and it is concluded that factors associated with differences in melting point rather than melting point of the fat itself are probably involved in heat resistance.

Introduction

Several writers have suggested that the action of heat on the lipids of the cell may be an essential feature of heat death (1, 6). This lipoid liberation theory of heat death is based on the observations that animals living at lower temperatures or raised at lower temperatures have fats with lower melting points than those living at higher temperatures or raised at higher temperatures. Pertinent references are cited in the reviews mentioned above. Heilbrunn (6) uses these observations in connection with his calcium release theory to explain heat death. According to this explanation the melting of the fats releases them from their combination with the proteins and calcium that are essential constituents of every cell membrane. Calcium released from the cell cortex to the interior of the cell brings about coagulation and death. The presence of fats of higher melting points in the cortex is responsible for increased resistance to heat. Acclimatization to heat is dependent upon the development of fats with higher melting points. Whether this explanation is accepted in detail or not, there is an apparent relation between the nature of fats and temperature at which the animal lives.

In this work an attempt has been made to test this theory by altering the melting point of the fats of goldfish and determining the effect of this treatment on the heat tolerance of the animals.

[1] *Manuscript received November 4, 1948.*

Contribution from the Department of Zoology, University of British Columbia, Vancouver, B.C., with financial assistance from the National Research Council of Canada and British Columbia Packers Limited.

[2] *Professor of Zoology and Fisheries.*

[3] *Graduate Student.*

Materials and Methods

These experiments are based on 265 goldfish (*Carassius auratus*) obtained from the Goldfish Supply Co., Stouffville, Ont. The fish ranged from 10 to 15 gm. in weight. They were divided into groups and fed diets containing 25% fat. Three different fats* were used: pilchard oil (iodine value 181.7), herring oil (iodine value 128.4), and lard (iodine value 66.2). As a base for these diets, 70% pablum (Mead Johnson Company of Canada, Ltd.) was used and the fat was bound to the pablum with lecithin (5%). The fat and lecithin were first dissolved separately in peroxide-free ether and then mixed. The solution of fat and lecithin was then poured into the pablum, mixed thoroughly, and the ether extracted *in vacuo* at 37° C. Fresh diets were prepared each week and stored in a refrigerator. The iodine values of the diets thus prepared were determined as follows: pilchard oil diet, 159; herring oil diet, 115; lard diet, 64. The stability of these diets was tested by determining the iodine values of the fats extracted from diets one week old and two weeks old. The change in value did not exceed one unit during this period.

The fish were fed, as a group, an amount of food equivalent to 0.3 gm. per fish per day. This amount was divided into three feedings and each feeding lasted approximately 30 min. Only a portion of the food was placed in the aquarium at one time so that the animals could eat as much as possible before it sank to the bottom and disintegrated. It was necessary to change the water and clean the aquaria once per week.

The fish were kept in glass aquaria. The water was aerated and the temperature maintained at 20° ± 0.5° C. To determine heat resistance, the fish were held at a constant high temperature. For this a large tank divided into three compartments with plastic netting was available. Thus, the three groups could be subjected to identical conditions for this test.

The iodine values of the oils were determined by the Wij's method. The extraction procedure consisted of macerating the fish in a Waring Blendor, drying it *in vacuo* at 45° to 55° C., and extracting it in an A.S.T.M. extraction apparatus with peroxide-free ethyl ether for two hours at 38° C. Following the extraction the ether was distilled off *in vacuo* at 38° C. and the oil dried in a vacuum desiccator.

Results

Effect of Dietary Fat on the Body Fats

The data are summarized in Table I and presented graphically in Fig. 1. The herring oil diet produced little change in the degree of unsaturation of the goldfish body fats.

* *The fish oils were supplied by Western Chemicals, Ltd., through the courtesy of Dr. W. Chalmers. The lard was procured from Burns and Co. Ltd. through Dr. B. E. Bailey. These were stored in the frozen condition at the Pacific Fisheries Experimental Station of the Fisheries Research Board of Canada. We are indebted to Dr. Bailey for numerous helpful suggestions.*

TABLE I

AVERAGE IODINE VALUES FOR GOLDFISH FED DIETS CONTAINING FATS OF DIFFERENT DEGREES OF UNSATURATION

Duration of feeding, days	Fish in each diet group	Iodine values		
		Pilchard oil diet (I.V. 159)	Herring oil diet (I.V. 115)	Lard diet (I.V. 64)
0	11*	—	113.5	—
6	6	122.3	115.5	93.4
12	6	124	112.9	90.4
21	6	126	115.6	—
29	8	128.2	112.6	88.4
32	4	133.4	112.9	86.5
34	6	136	111.4	89.2
37	6	136.1	113.1	86.4
46	6	137.1	113.2	91.38
55	8	137	110	85.8

* *Entire sample.*

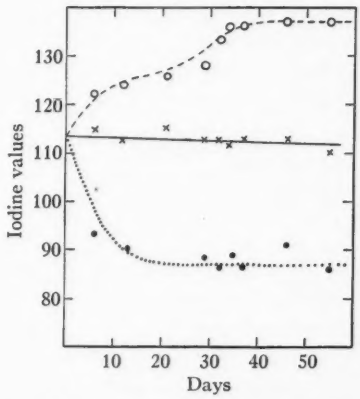

FIG. 1. *Changes in degree of unsaturation of body fats of goldfish fed pilchard oil (broken line), herring oil (solid line), and lard (dotted line).*

This was expected from a comparison of the value for prefed fish (113.5) with the iodine value of this diet (115). The pilchard oil diet and the lard diet produced very definite changes in the degree of unsaturation of the goldfish fats. The change was more rapid and reached its maximum value more quickly in the case of the solid fat (12 days as compared to 34 days). In both cases the value became constant at about 54% of the maximum theoretical change possible if the fats of the fish were entirely altered to those of the diet.

Effect of Dietary Fat on Heat Tolerance

The histogram of Fig. 2 shows the temperature tolerance of the different groups as measured by the time required to kill 50% of a sample of 90 goldfish (30 from each diet group) at a temperature of 36° C. Three of the fish on

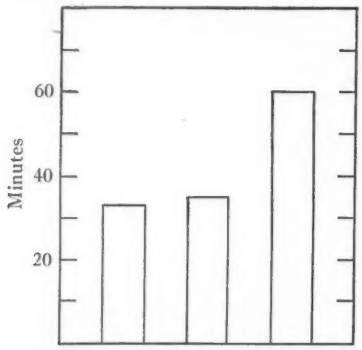

FIG. 2. *Time required to kill 50% of a sample of goldfish fed on three diets differing in fat content. Diets from left to right are pilchard oil, herring oil, and lard.*

the lard diet lived 24 hr. (and longer) at 36° C. The maximum survival time for fish on the herring oil diet was 80 min.; for those on the pilchard oil diet 137 min. These fish had been fed on the high-fat diets for 46 days and consequently the change in body fats had reached a maximum. The contrast in heat tolerance between the solid fat (lard) and the liquid fats (herring or pilchard oil) is evident. Moreover, the order of heat resistance is that which would be expected if heat tolerance is related to melting point of fats. However, the correlation between iodine value (hence melting point) of the body fats and heat tolerance is not precise. The iodine values form an evenly graduated series (85, 110, 137) but there is a very small difference between the lethal times of the fish fed herring oil and pilchard oil (35 min. compared to 33.5 min.).

In Fig. 3 the data for this experiment have been treated in another way. The lines show the rates at which the fish died during the first 75 min. at 36° C. The order again is the order anticipated if there is a relation between melting point of fats and heat tolerance. However, the fiducial limits (99% level of confidence) of the regression coefficients show that there is no statistical difference between the behavior of the samples on the two fish-oil diets but that the group on the solid diet (lard) is distinct.

A preliminary series of temperature tolerance tests involving approximately 100 fish demonstrated essentially the same features as shown above. These results are not presented in detail since the fish fed the pilchard oil diet had not been fed long enough to produce the maximum change in their body fats. The lethal times (50% level) and rates of dying for the goldfish fed lard and herring oil were almost identical while the fish fed pilchard oil survived much

longer than those of the experiments above. This difference is readily explained by the fact that the pilchard oil diet had produced only a partial change in the goldfish fats at the time of the tests.

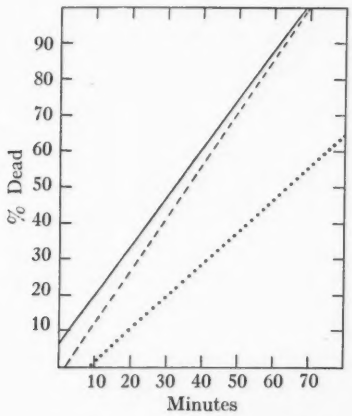

FIG. 3. *Regression lines to show the rate at which goldfish fed three different diets were killed at 36° C. Solid line—herring oil diet ($b = 6.82 \pm 1.08$); broken line—pilchard oil diet ($b = 7.34 \pm 1.32$); dotted line—lard diet ($b = 4.42 \pm 0.293$).*

Discussion

It is evident that the degree of unsaturation of the body fats of the goldfish can be modified by dietary fat. This fact has been frequently demonstrated for mammals (9, 10) and was found by Lovern (10) to be true for the eel (*Anguilla vulgaris*) if the diet was high in fat (20.7%). The present experiments show that the fats of goldfish are altered more readily with lard than with pilchard oil although the degree of unsaturation of these two fats differ about equally from the goldfish fat prior to feeding. Deposition of dietary fat in mammals likewise varies with the nature of the fat (9). In the present experiments, the maximum change produced in the goldfish is only about 54% of the theoretical.

Further, it is apparent that the heat tolerance of goldfish can be modified by dietary fat. The greatest effect, obtained with the lard diet, corresponds to an acclimation temperature of about 24° C. or a change in acclimation temperature equivalent to 4° C. (5).

The data demonstrate a general relationship between degree of unsaturation of the body fats and heat tolerance. If, however, heat tolerance is dependent upon the melting point of the body fats there should be a strict correlation between these two factors. This is not apparent. The pilchard oil and herring oil groups behave very similarly. If melting point is the important factor, it must be concluded that the difference between the melting points of the fats of fish fed herring and pilchard oil is not great enough to change the heat

tolerance. It seems more likely that some other property of these fats is involved. These analyses are based on total ether extract. The nature of the phospholipid fats or other ether soluble materials may be more important. Variations in the phospholipid fat have been demonstrated for poikilothermic animals in relation to temperature of the environment (12, 7). Another possibility is that the different fats bring about changes in the water content of the tissues (11) or permeability of the cell membranes to water (2) and thus affect the heat tolerance.

The failure to show a precise correlation between the melting point of the body fats and heat resistance is in line with experiments on temperature acclimation of goldfish (8) and the breeding of blowflies at different temperatures (4).

In connection with the present experiments it would be interesting to know the lethal temperature relations of pilchard (*Sardinops caerulea*) and herring (*Clupea pallasii*) since the former is a more southerly fish although its fat has a lower melting point. This is contrary to the expectation from findings for many other species where the animals living in the lower temperature environment have the fats with lower melting point (references previously cited).

As yet the temperature relations of these groups of experimental fish have not been completely worked out. Observations on the behavior of the fish when subjected to high temperature indicate that the herring and pilchard oil diets had definite effects on the fish that cannot be demonstrated by lethal times and rates. Further experiments are in progress. It may be mentioned, however, that the initial reactions of fish fed on herring oil are more violent than those fed on pilchard oil. In several cases fish fed herring oil died immediately on being placed in the warm water. This phenomenon was not observed with the other fish. The herring oil diet appears to produce nervous symptoms in the fish even at room temperature. Brown (3) observed nervous symptoms in pigs fed on diets containing 14% menhaden oil although the fish oil acids had not accumulated in the brain. From these observations it is felt that the herring and pilchard oil diets have produced changes in temperature relationships not demonstrated by the lethal times.

References

1. BĚLEHRÁDEK, J. Temperature and living matter. Protoplasma-monographien 8, Berlin. 1935.
2. BROOKS, S. C. and BROOKS, M. M. The permeability of living cells. Protoplasma-monographien 19, Berlin. 1941.
3. BROWN, J. B. The nature of the highly unsaturated fatty acids stored in the lard from pigs fed on menhaden oil. J. Biol. Chem. 90 : 133-139. 1931.
4. FRAENKEL, G. and HOPF, H. S. The physiological action of abnormally high temperatures on poikilothermic animals. I. Temperature adaptation and degree of saturation of the phosphatides. Biochem. J. 34 : 1085-1092. 1940.
5. FRY, F. E. J., HART, J. S., and WALKER, K. F. Lethal temperature relations for a sample of young speckled trout, *Salvelinus frontinalis*. Appendix. Univ. Toronto Studies, Biol. Ser. 54. Pub. Ont. Fisheries Research Lab. 66 : 9-35. 1946.
6. HEILBRUNN, L. V. An outline of general physiology. 2nd ed. W. B. Saunders Company, Philadelphia. 1943.

7. HOPF, H. S. The physiological action of abnormally high temperatures on poikilothermic animals. 3. Some changes occurring in the phosphorus distribution of the haemolymph of insects under the influence of abnormally high temperatures. Biochem. J. 34 : 1396-1403. 1940.

8. HUNTER, J. G. The change in the degree of unsaturation of body fats during acclimation of goldfish (*Carrassius auratus*) to high temperature. M.A. thesis, University of British Columbia. (Unpub.) 1948.

9. LONGENECKER, H. E. The formation of animal body fat. Biol. Symposia, 5 : 99-115. 1941.

10. LOVERN, J. A. Fat metabolism in fishes. XIII. Factors influencing the composition of the depot fat of fishes. Biochem. J. 32 : 1214-1224. 1938.

11. MAYER, A. and SCHAEFFER, G. Recherches sur les constantes cellulaires teneur des cellules en eau. II. Rapport entre la teneur des cellules en lipoïdes et leur teneur en eau. J. physiol. et path. gén. 16 : 23-38. 1914.

12. TERROINE, E. F., HATTERER, C., and ROEHRIG, P. Les acides gras des phosphatides chez les animaux poikilothermes, les végétaux supérieurs et les microorganismes. Bull. soc. chim. biol. 12 : 682-702. 1930.

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